GENETIC CHARACTERIZATION OF *BEAUVERIA BASSIANA* ASSOCIATED WITH FIRE ANTS, *SOLENOPSIS* SPP., AND OTHER INSECTS

BY

YONG ZENG

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1999

ACKNOWLEDGMENTS

I would like first to thank Dr. J. L. Stimac for his guidance, understanding, encouragement and support through all stages of my graduate studies. This project and I benefited immensely from his trust, perseverance, and his emphasis on objective-oriented focusing and systematic thinking. Special appreciation goes to Dr. H. C. Kistler, who greatly facilitated this project through the kind use of his laboratory and his expertise. I thank him for his understanding and constant encouragement. I am very grateful to Dr. J. E. Maruniak for encouragement and the help he offered at the start of this project and also for the insightful advice he has given throughout. I greatly want to thank Dr. J. W. Kimbrough and Dr. J. L. Nation for their valuable input into my graduate study, in particular this project.

Also, I would like to thank and acknowledge the following: Dr. U. L. Rosewich for her enormous assistance in the research; Dr. A. Jeyaprakash for his generous technical support and essential suggestions; Dr. D. G. Boucias for his helpful insights and support during the work; L. A. Wood for her substantial help on this project; and R. E. Pettway for his enthusiasm and valuable skills. Many thanks must also go to U. Benny and Dr. A. Garcia-Maruniak for their tremendous technical support. My thanks go out to Dr. H. G. Hall, Dr. R. M. Pereira, Dr. B. C. Larson-Vasquez, Dr. M. J. Parsons, H. L. Kons Jr. and the friends I have made at the University of Florida. Finally, I thank my parents, my sister Yvonne and my wife Jeniffer for their support, encouragement, and love.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS....ii

LIST OF TABLES....v

LIST OF FIGURES....vii

ABSTRACT....ix

CHAPTERS

1 INTRODUCTION....1

Biology of *Beauveria bassiana*....1
  Classification....1
  Genetic System....4
  Natural Occurrence....4
Beauveria bassiana as a Biological Control Agent....5
  History and Status....5
  Challenges and Potentials....7
Genetic Diversity in *Beauveria bassiana*....8
  Protein and Isoenzyme Analyses....8
  Genomic DNA Analyses....9
Objectives....11

2 DNA POLYMORPHISMS IN *BEAUVERIA BASSIANA*: RFLP PROBE DEVELOPMENT AND SEQUENCE ANALYSIS OF THE ITS AND 5.8*s* rRNA GENE REGION....13

Introduction....13
Materials and Methods....17
  Fungal Cultures and *B. bassiana* Isolates....17
  DNA Extraction....17
  PCR-amplification, Sequence and Restriction Analysis of the ITS and 5.8*s* rRNA Gene Region....19
  Constructing and Screening a Genomic Library....21
  Enzyme Digestion, Electrophoresis, Southern Blotting and Probe Choice....22
Results....23
  Sequencing and Restriction Analysis of the ITS and 5.8s Gene region....23

    Isolation and Evaluation of Recombinant Clones....................23
    DNA Polymorphisms by Using Homologous RFLP Probes....................29
    DNA Polymorphisms by Using Heterologous DNA Probes....................33
  Discussion....................36

3 GENETIC DIVERSITY IN *BEAUVERIA BASSIANA* ISOLATES ASSOCIATED WITH FIRE ANTS, *SOLENOPSIS* SPP., AND OTHER HOST SPECIES....................41

  Introduction....................41
  Materials and Methods....................43
    B. bassiana Isolates....................43
    DNA Extraction, Choice of Restriction Enzymes and Probes....................43
    Data Analysis....................48
  Results....................51
    Genetic Diversity among *B. bassiana* Isolates Associated with *Solenopsis* spp....................51
    Comparison of Genetic Variation between Isolates from *Solenopsis* spp. and Isolates from Other Host Species....................53
  Discussion....................59

4 GENETIC POPULATION STRUCTURE IN *BEAUVERIA BASSIANA* ASSOCIATED WITH FIRE ANTS, *SOLENOPSIS* SPP....................66

  Introduction....................66
  Materials and Methods....................68
    B. bassiana Isolates....................68
    DNA Extraction and Choice of Probes....................72
  Results....................72
    Genetic Variation among *B. bassiana* Isolates Associated with *Solenopsis* spp....................72
    B. bassiana Isolates from *Solenopsis* spp. in the United States....................78
  Discussion....................81

5 SUMMARY AND CONCLUSIONS....................86

LIST OF REFERENCES....................92

BIOGRAPHICAL SKETCH....................111

LIST OF TABLES

Table Page

2-1. *Beauveria bassiana* isolates evaluated for DNA polymorphisms based on sequence analysis of the ITS and 5.8s rRNA gene region or RFLP probes........18

2-2. Restriction enzyme analysis of the ITS and 5.8s rRNA gene region 28

2-3. RFLP banding patterns among isolates from fire ants, GHA and Naturalis by single and low copy homologous RFLP probes.....32

3-1. *Beauveria bassiana* isolates from fire ants and nest soil for testing single locus and repetitive probes........44

3-2. Genetic variation of *Beauveria bassiana* isolates from *Solenopsis* spp. based on repetitive probe 133 and the telomeric probe in conjunction with *Pst* I........45

3-3. DNA banding patterns generated by repetitive probes with *Hin*d III-digested DNA of *Beauveria bassiana* isolates collected from different hosts........46

3-4. Comparison of DNA pattern banding types generated by single-locus and repetitive probes with *Hin*d *III*-digested DNA of *Beauveria bassiana* isolates from fire ants and ant nest soil........47

3-5. Genetic homogeneity among *Beauveria bassiana* isolates from *Solenopsis* species digested with *Eco* RI, *Hin*d III and *Pst* I........49

3-6. Genetic variation of *Beauveria bassiana* isolates from *Solenopsis* spp. digested with *Bam* H I, *Eco* RV and *Hae* III and hybridized to repetitive probe 133 and the telomeric probe........50

4-1. Genetic variation among *Beauveria bassiana* isolates collected from *Solenopsis invicta* in Cuiaba, Mato Grosso, Brazil in 1986 and 1987.........69

4-2. Genetic variation among *Beauveria bassiana* isolates from *Solenopsis* species or nest soil in different locations........70

4-3. *Beauveria bassiana* isolates collected in Gainesville, Florida......................71

4-4. *Beauveria bassiana* isolates collected from *Solenopsis xyloni* in Bakersfield, California......................73

LIST OF FIGURES

Figure Page

2-1. Sequence alignment of the ITS and 5.8*s* rRNA gene regions for *Beauveria bassiana* isolate 447 and 14 other isolates obtained from Genbank............24

2-2. DNA fingerprinting profile of *Beauveria bassiana* isolates by using probe 133. Genomic DNA was digested with *Hin*d III (A) and *Pst* I (B)...............30

2-3. DNA fingerprinting profile of *Beauveria bassiana* isolates by using probe 205. Genomic DNA was digested with *Hin*d III (A) and *Pst* (B)................31

2-4. DNA fingerprinting profile of *Beauveria bassiana* isolates by using a telomeric probe from *Fusarium oxysporum.* Genomic DNA was digested with *Hin*d III (A) and *Pst* I (B)..34

2-5. DNA banding patterns of *Beauveria bassiana* isolates by using a mitochondrial probe from *Fusarium oxyporum.* Genomic DNA was digested with *Eco* RI (A) and *Hin*d III (B) 35

3-1. DNA fingerprinting profile of *Beauveria bassiana* isolates collected from *Solenopsis* spp. Genomic DNA was digested with *Bam* HI and hybridized to a telomeric probe from *Fusarium oxysporum*.........................52

3-2. DNA fingerprinting profile of *Beauveria bassiana* isolates collected from *Solenopsis* spp. and other insects. Genomic DNA was digested with *Hind* III and hybridized to a telomeric probe from *Fusarium oxysporum*..54

3-3. Dendrogram showing the genetic relationships among *Beauveria bassiana* isolates. Cluster analysis was performed by the unweighted pair-group method with arithmetic mean (UPGAM). Similarity coefficients were based on 59 bands generated by the telomeric probe hybridized to *Hind* III digested DNA..55

3-4. Dendrogram showing the genetic relationships among *Beauveria bassiana* isolates. Cluster analysis was performed by the unweighted pair-group method with arithmetic mean (UPGAM). Similarity coefficients were based on 45 bands generated by probe 133 hybridized to *Hind* III digested DNA...56

3-5. Dendrogram showing the genetic relationships among *Beauveria bassiana* isolates. Cladistic approach was performed by parsimony analysis with data from the telomeric probe. Numbers below branches represent bootstrap value based on 500 replicates. Only values greater than or equal to 50% are shown....56

3-6. Dendrogram showing the genetic relationships among *Beauveria bassiana* isolates. Cladistic approach was performed by parsimony analysis with data from probe 133. Numbers below branches represent bootstrap value based on 500 replicates. Only values greater than or equal to 50% are shown....57

4-1. Example of DNA banding patterns of *Beauveria bassiana* isolates collected from *Solenopsis invicta* in Cuiaba, Mato Grosso, Brazil in 1986 (A and C) and 1987 (B). Genomic DNA was digested with *Hin*d III and hybridized to a telomeric probe from *Fusarium oxysporum* (A and B) and probe 133 (C)....74

4-2. Example of DNA banding patterns of *Beauveria bassiana* isolates collected from *Solenopsis* spp. in different locations. Genomic DNA was digested with *Hin*d III and hybridized to a telomeric probe from *Fusarium oxysporum*....76

4-3. Example of DNA banding patterns of *Beauveria bassiana* isolates collected from *Solenopsis* spp. in different locations. Genomic DNA was digested with *Hin*d III and hybridized to probe 133....77

4-4. Example of DNA banding patterns of *Beauveria bassiana* isolates collected from *Solenopsis quinquecuspis*, *S. saevissima* and *Solenopsis* spp. Genomic DNA was digested with *Hin*d III and hybridized to a telomeric probe from *Fusarium oxysporum*....79

4-5. Example of DNA banding patterns of *Beauveria bassiana* isolates collected from *Solenopsis invicta* or nest soil in Florida (A) and from *S. xyloni* in California (B). Genomic DNA was digested with *Hind* III and hybridized to a telomeric probe from *Fusarium oxysporum*....80

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

GENETIC CHARACTERIZATION OF *BEAUVERIA BASSIANA* ASSOCIATED WITH FIRE ANTS, *SOLENOPSIS* SPP., AND OTHER INSECTS

By

Yong Zeng

August 1999

Chairman: Jerry L. Stimac
Major Department: Entomology and Nematology

Beauveria bassiana isolate 447, obtained from *Solenopsis invicta* in Brazil, has been developed as a microbial control agent against fire ants in the United States. To genetically characterize isolate 447 and other *B. bassiana* isolates, including commercial product isolates, various molecular markers were evaluated. The limited DNA sequence heterogeneity of the ribosomal internal transcribed spacers (ITS) and 5.8*s* rRNA gene region was considered insufficient to efficiently discriminate among isolates by using restriction analysis. Two homologous repetitive DNA sequences (probe 133 and probe 205) and two heterologous probes (a telomeric probe and a mitochondrial probe from *Fusarium oxysporum)* were able to distinguish isolate 447 from the other commercial product isolates, GHA and Naturalis.

None of 17 isolates from 12 non-fire ant species that were tested with probe 133 and the telomeric probe were similar to isolates from *Solenopsis* spp. Some *B. bassiana* genotypes from *Solenopsis* spp. appear to belong to a *Solenopsis* genus-preferential clone lineage. Genotype similarity in the lineage suggests a co-evolutionary history between this *B. bassiana* lineage and *Solenopsis* spp. Genetic diversity analysis with probe 133, the telomeric probe and single locus probes indicates that asexual reproduction is a predominant reproduction mode among *B. bassiana* isolates associated with *Solenopsis* spp. Parsimony analysis suggests that *B. bassiana* may not be a species with exclusively clonal reproduction.

Probe 133 and the telomeric probe can be used to evaluate the ecological adaptability of isolate 447 as a biological control agent against fire ants. When *B. bassiana* isolates collected from four *Solenopsis* species in ten locations were examined, multiple genotypes were commonly found among isolates collected from the same species at the same location. One genotype was predominant regardless of the *Solenopsis* species or geographic location. This indicates that this predominant genotype, which also includes isolate 447, can survive in a wide range of ecological conditions. After isolate 447 was released at treatment sites in Florida and California, isolates with the same genotype of isolate 447 were recovered one year later, indicating the successful colonization of an exotic isolate released in the United States.

CHAPTER 1

INTRODUCTION

Biology of *Beauveria bassiana*

Classification

At the beginning of this century, all *Beauveria* species were placed in the genus *Botrytis* in Europe and in the genus *Sporotrichum* in the United States. Vuillemin (1912) was the first to separate the genus *Beauveria* from *Botrytis* (*Sporotrichum),* based on the geniculate fertile portions of conidiogenous cells. Petch (1926) analyzed cultural characters as taxonomic criteria for species determination and grouped *Beauveria* isolates into two species complexes based on spore shape. Later, after the genus *Tritirachium* was described to have the same characters as *Beauveria* (Limber 1940), Macleod (1952) found other taxonomic characters to distinguish *Tritirachium* from *Beauveria.* Macleod (1952) also placed fourteen contemporary *Beauveria* species into either *Beauveria bassiana* (Balsamo) Vuillemin (globose or oval conidia) or *Beauveria tenella* (Delacroix) Siemaszko (ellipsoidal conidia). De Hoog (1972) renamed *B. tenella* as *B. brongniartii* and added *B. alba* as a new species to the genus *Beauveria.* More species were added to the genus (De Hoog 1972; Samson and Evans 1982), including species the genus *Tolypocladium*, which was integrated into *Beauveria* based on numerous morphological criteria (Von Arx 1986).

The problem of delineating species in the genus *Beauveria* based on morphological criteria accelerated the development of more objective taxonomic criteria provided by biochemical and molecular techniques (Samson 1995). Based on serological characteristics, *B. bassiana* and *B. brongniartii* were found to display unique serotypes, although these two species also possessed some of the same antigens (Shimizu and Aizawa 1988). The genus *Beauveria* was more clearly characterized with 64 morphological and biochemical characters (Mugnai et al. 1989). Two isolates of *Tolypocladium cylindrosporum* clustered together and were found to be distinct from *Beauveria*. Hydrolytic enzyme, API ZYM and esterase patterns used in conjunction with morphological characters confirmed that spore shape rather than cultural characteristics was the more useful for species determination. Six species of *Beauveria* (*B. alba, B. amorpha, B. bassiana, B. brongniartii, B. velata, and B. vermiconia*) were recognized based upon a unweighted pair group arithmetic mean method (UPGMA) dendrogram and principal components analyses. Conversely, *B. densa* and *B. stephanoderis* were considered aberrant strains of *B. bassiana.* Furthermore, rRNA sequence comparison of two highly variable domains in the 28S region confirmed phylogenetic differences between *Beauveria* and *Tolypocladium* (Rakotonirainy et al. 1991). Using cluster analysis with data from four isoenzymes, St Leger et al. (1992) were able to distinguish the following species: *B. brongniartii, B. caledonica, B. vermiconia* and *Tolypocladium cylindrosporum*. Among 138 *Beauveria* isolates tested, *B. bassiana, B. brongniartii* and *B. amorpha* were clustered together. As the one tested isolate of *B. caledonica* was placed within the core group of *B. bassiana, B. caledonica* was suggested to be a rare diploid strain of *B. bassiana.*

Among DNA techniques, RAPDs distinguished *B. bassiana* from other entomopathogenic fungi (Bidochka et al. 1994). In addition, *B. brongniartii* and *B.*

bassiana were separated by ribosomal internal transcribed spacers (ITS) regions analysis with PCR and RFLPs (Neuveglise et al. 1994). Hegedus and Khachatourians (1993a) developed DNA probes from a genomic library of *B. bassiana* and used four probes to distinguish *B. bassiana* from *B. brongniartii, B. caledonica, B. densa, T. cylindrosporum, T. nivea* and other related species. Two of these *B. bassiana*-DNA probes produced identical hybridization banding patterns for both *B. densa* and *B. bassiana* under low stringency conditions. Therefore it was suggested that *B. densa* was an aberrant strain of *B. bassiana* (Mugnai et al. 1989).

When DNA was digested with *Eco* RI and hybridized to five *B. bassiana* mitochondrial (mt) probes, 15 *B. bassiana* isolates could be differentiated from *B. brongniartii* and other entomopathogenic fungi (Hegedus and Khachatourians 1993b). Conversely, the same probe-enzyme combinations generated identical or very similar hybridization patterns for isolates of *B. bassiana, B. caledonica* and *B. densa,* again indicating the close phylogenetic relationships among these three species as other studies had done (Mugnai et al. 1989; St Leger et al. 1992). The combination of *Hind* III and one of the five mitochondrial probes eventually distinguished *B. bassiana, B. caledonica* and *B. densa* from each other.

More interestingly, Hegedus and Khachatourians (1996) designed a set of PCR primers based on sequence analysis of one DNA probe from *B. bassiana*. Although similar-sized PCR products were generated for most tested isolates of *B. bassiana, B. brongniartii, B. caledonica* and *B. densa,* RFLPs, sequence analysis and single-strand conformation polymorphisms (SSCPs) of PCR products differentiated among these *Beauveria* species. Furthermore, by exploiting the sequence heterogeneity of the PCR products, another set of primers was successfully developed to specifically amplify *B. bassiana*.

Genetic System

Beauveria and *Tolypocladium* had been suggested to share a common teleomorph genus, *Cordyceps,* due to the molecular similarities of the two species (Mugnai et al. 1989). However, there is no evidence that *B. bassiana* reproduces sexually. If sexual reproduction is found in *B. bassiana*, the species will belong to *Cordyceps*. Though parasexual recombination has occurred after protoplast fusion in the laboratory (Kawanmoto and Aizawa 1986; Shimizu 1987; Paccola-Meirelles and Azevedo 1991), parasexuality has not been observed to occur in natural populations. Electrophoretic karyotyping of *B. bassiana* revealed seven to eight chromosomes, resulting in a genome size of 34.3 to 44.1 Mb (Pfeifer and Khachatourians 1993; Viaud et al. 1996).

Natural Occurrence

The white muscardine fungus, *B. bassiana,* has been extensively studied since it was first reported as a pathogen of the silkworm, *Bombyx mori* L., by Agostino Bassi (1773-1856). The worldwide host range of *B. bassiana* includes over 200 different insect species belonging to nine orders, mainly to Lepidoptera and Coleoptera (Feng et al. 1994). New host species are constantly added. For example, *B. bassiana* has been recently isolated from *Hypera brunneipennis* (Johnson et al. 1984), *Musca domestica* (Steinkraus et al. 1990), *Alphitobius diaperinus* (Steinkraus et al. 1991), *Phyllophaga anxia* (Poprawski and Yule 1991), and *Lygus lineolaris* (Steinkraus and Tuqwell 1997).

Beauveria bassiana as a Biological Control Agent

History and Status

Designing standardized bioassays for specific target pests is critical for accurately measuring the virulence of a particular strain of *B. bassiana* and evaluating its performance in field applications. Target pests for which standardized bioassays have been developed include *Solenopsis invicta* (Pereira et al. 1993a and 1993b; Stimac et al. 1993a and 1993b), *Bemisia argentifolii* (Landa et al. 1994) and *Diuraphis noxia* (Vandenberg 1996). Also, standardized bioassays have allowed assessing attenuation of virulence due to repeated subculture (Ssamsinakova and Kalaova 1983; Hall 1982b; Hayden et al. 1992).

During strain screening for potential target pests, it has been found that isolates of *B. bassiana* usually have higher virulence to their original host species or closely related species than to other insect species (Feng et al. 1994). Therefore, most approaches have focused on evaluating the efficacy of host-derived isolates of *B. bassiana* against their original host species (Anderson and Roberts 1983; Stimac et al. 1987; Prior et al. 1988; Moore and Erlandson 1988; Feng et al. 1990b; Bing and Lewis 1991; Poprawski and Yule 1991; Goettel 1992; Geden et al., 1995; Varela and Morales 1996; Vandenberg 1996).

In addition, several studies also evaluated specific strains of *B. bassiana* for their effectiveness against insect species other than their original host in order to assess the potential for expanding market opportunities (McCoy et al. 1985; Poprawski et al. 1985; McDowell et al. 1990; Feng and Johnson, 1990; Feng et al. 1990; Wright and Chandler 1991, 1992; Wright 1992; Khachatourians 1992; Eyal et al. 1994; Kaaya et al. 1995, 1996; Bateman et al. 1996; Jones et al. 1996; Castineiras et al. 1996; Vandenberg 1996; Steinkraus and Tuqwell 1997; Wraight et al. 1998). It has been found that host range can be enlarged by using bioassays. Most *B. bassiana* isolates are pathogenic to many target insects when inoculum levels are relatively high (Ferron and Robert 1975; Fargues 1976; Feng and Johnson 1990a). Since over 700 species of invertebrates are susceptible to *B. bassiana* (Goettel et al. 1995), one main concern is the effect of *B. bassiana* on nontarget insect species (Goettel et al. 1990). Some studies evaluated the susceptibility of the insect predator *Coleomegilla maculata* to *B. bassiana* isolates (Lord et al. 1988; Todorova et al. 1994, 1996; Pingel and Lewis 1996).

The pathogenic capabilities of *B. bassiana* were shown to be dependent on both virulence and aggressiveness (Riba et al. 1982). In addition, quantitative bioassays demonstrated not only differential virulences among *B. bassiana* strains but also positive correlation between mortality of targeted hosts and conidial concentration (Ferron and Robert 1975; Hall 1982a; and Feng et al. 1990b).

In commercial applications as a biological control agent, *B. bassiana* has been successfully used to control European corn borers and various forest and crop pests in China (Feng et al. 1994). In countries of the former U. S. S. R. and in eastern Europe, *B. bassiana* has been used to control Colorado potato beetles and codling moths (Ferron 1981; McCoy et al. 1988)

In the U.S., *B. bassiana* was first experimentally developed for use against Lygus bugs by Nutrilite Products Inc. in 1962 (Dunn and Mechalas 1963; Ignoffo et al. 1979). During the 1980s, Abbott Laboratories developed a wettable formulation of *B.*

bassiana conidia for use against Colorado potato beetles (Campbell et al. 1985; Hajek et al. 1987; Anderson and Roberts 1983; Anderson et al. 1988, 1989). In the early 1990s, Naturalis ® was developed and registered for control of boll weevils and whiteflies (Wright and Chandler 1991, 1992a, 1992b, and 1993). More recently, Mycotech registered Mycotrol GHA ® as an oil flowable for use against grasshoppers and BotaniGard ® as a wettable powder for use against whiteflies, thrips, and aphids (Johnson and Goettel 1993; Goettel et al. 1995; Wraight et al. 1996, 1998). Isolate 447 of *B. bassiana* obtained in Brazil from *Solenopsis invicta* has been tested for controlling the red imported fire ant, *S. invicta* in Florida and the Southern fire ant, *S. xyloni*, in California (Stimac et al. 1987; Stimac and Alves 1994; Oi et al. 1994). Additional studies have indicated the potential for developing this particular isolate against household ants, cockroaches and termites.

Challenges and Potentials

Like other mycopesticides, several factors have limited the success of developing *B. bassiana* isolates as biological control agents (McCoy et al. 1988; Leathers et al. 1993). First, the relatively high cost for mass production and formulation limits their market potential (Feng et al. 1994). Second, as *B. bassiana* conidia are sensitive to UV light, field applications may lead to inconsistent efficacy (Studdert and Kaya 1990; Roberts and Hajek 1992; Inglis et al. 1997). Third, an incomplete understanding of the biology of *B. bassiana,* especially regarding host-pathogen interactions, has lead to low efficacy to control target pests and to a relatively narrow host range compared to chemical pesticides (McCoy et al. 1988; Hajek et al 1994). Finally, the lack of knowledge of the evolutionary genetics of *B. bassiana* is an obstacle for improving strain typing and strain selection. Strain typing is critical for evaluating introduced strains versus indigenous strains, for monitoring the fate of introduced strains in the environment, for controlling product quality and for protecting the product from patent infringement.

Advances for improving *B. bassiana* as a mycopesticide are promising. New approaches for mass production will improve the marketing potential of *B. bassiana* products (Alves and Pereira 1989; Pereira and Roberts 1990; Bradley et al. 1992). Continued efforts to improve formulations will enhance field performances of *B. bassiana*, especially related to targeting insects and its persistence in the field (Stimac and Alves 1994; Inglis et al. 1996; Jaronski 1997).

Improving the efficacy of mycopesticides also should focus on the identification of highly virulent strains and on genetic manipulations for strain improvements (Kistler 1991; Roberts and Hajek 1992). Screening has lead to the identification of more efficient strains (Stimac et al. 1987; Stimac and Alves 1994). Increase in virulence by protoplast fusion in *Beauveria* has been studied extensively (Pfeifer and Khachatourians 1992; Couteaudier et al. 1996; Viaud et al. 1998).

Genetic Diversity in *Beauveria bassiana*

Protein and Isoenzyme Analyses

Isoenzyme analysis reveals genetic variation among *B. bassiana* isolates collected from *Adelphocoris lineolatus* in one region in Italy. In contrast, isolates collected from *Lugus rugulipennis* in one French region were genetically homogenous (Riba et al. 1986). In a similar study, isolates collected from *Sitona humeralis* in different regions of France showed the same α-esterase isoenzyme profile, whereas isolates collected from *S. discoideus* in different regions in France and Morocco were highly variable (Poprawski et al. 1988). Also, some biochemical homogeneity among *B. bassiana* isolates from related hosts and from the same geographical areas was demonstrated by API ZYM and esterase patterns (Mugnai et al. 1989). Other biochemical studies among *B. bassiana* isolates from *Ostrinia nubilalis* and *Hypotherenmus hampei* lead to the conclusion that *B. bassiana* isolates from the same insect species are sometimes genetically homogeneous (Bridge et al. 1990). On the other hand, isoenzyme profiles of 138 isolates of *B. bassiana* broadly representing geographical and host ranges for the species revealed high levels of genetic diversity and no host specificity or geographical grouping (St. Leger et al. 1992). Three geographically widespread genotypes were detected, accounting for the majority of surveyed isolates. This study suggested that a few clones dominate the population of *B. bassiana* worldwide.

Genomic DNA Analyses

PCR and RFLP analyses of the ITS and 5.8*s* rRNA gene region showed that 28 isolates of *B. brongniartii* displayed high levels of polymorphism among isolates (Neuveglise et al. 1994). In the same study, only two isolates of *B. bassiana* were analyzed. These two isolates had the same restriction patterns. However, in another study with two isolates of *B. bassiana*, the nucleotide sequences in the 5.8s rDNA coding gene and ITS regions displayed heterogeneity (Shih et al. 1995).

Six *B. bassiana* isolates collected from different geographical regions and hosts or substrates were found to contain some polymorphisms based on four DNA probes, but these probes were not used to analyze additional isolates (Hegedus and Khachatourians 1993a). Another study with three RAPD primers distinguished three isolates of *B. bassiana* collected from different geographical regions and hosts (Bidochka et al. 1994).

Seven *B. bassiana* isolates collected from *Ostrinia nubilalis* in different geographical regions in Europe formed a distinct subspecific group based on vegetative compatibility grouping (VCG) (Couteaudier and Viaud 1997). Four isolates from France and five isolates from Morocco, all collected from *Sitona discoideus,* belong to different VCGs. With a heterologous telomeric DNA probe, seven *B. bassiana* isolates collected from *O. nubilalis* showed very similar RFLP banding patterns. Variation between isolates was restricted to one or two bands. Similarly, all isolates from *S. discoideus* were highly variable with telomeric DNA probe analysis. The results suggested that variation by telomeric DNA probe analysis was correlated with VCG groupings.

Homologous DNA probe analysis with six random and two known sequences placed twelve isolates from *O. nubilalis* and *O. furnacalis* into distinct groups regardless of geographical origin (Maurer et al. 1997a). Futhermore, ten isolates from *O. nubilalis* had the same RFLP banding pattern. Also, RAPD analysis with four primers agreed with the RFLP analysis in this study. In another study, pulsed-field gel eletrophoresis (PFGE) indicated that chromosome length polymorphisms exist in seven *B. bassiana* isolates collected from different hosts, but no karyotype polymorphisms were observed among three isolates from one insect host, *O. nubilalis* (Viaud et al. 1996).

Objectives

B. bassiana isolate 447, obtained in Brazil from *S. invicta,* has been evaluated as a microbial control agent in the United States against fire ants such as *S. invicta* and *S. xyloni*. In this study, to genetically characterize isolate 447 and other *B. bassiana* isolates, including commercial product isolates, DNA sequence analyses of the ITS and 5.8*s* rRNA gene region were done for isolate 447 and 14 other *B. bassiana* isolates. Restriction analysis of the ITS and 5.8*s* rRNA gene region was conducted for nine additional isolates. Also, homologous repetitive probes, heterologous telomeric and mitochondrial probes were developed and utilized to genetically characterize *B. bassiana* isolates (Chapter 2).

To determine host-genotype relationships of *B. bassiana* isolates associated with *Solenopsis* spp. and other host species, single locus DNA probes and repetitive DNA probes were utilized to analyze genetic diversity among isolates collected from *Solenopsis* spp. and other host species. Furthermore, phylogenetic analysis was performed to characterize host-genotype relationships (Chapter 3).

To evaluate the ecological adaptability of isolate 447, a telomeric probe and a repetitive DNA probe were used to describe genetic population structure of *B. bassiana* associated with *Solenopsis* spp. in a wide range of ecological conditions in South America. Detailed population structure analysis was conducted at one location in two consecutive years. *B. bassiana* isolates collected from release sites in Florida and California were examined to evaluate colonization of an exotic isolate released in the United States (Chapter 4).

In the final chapter (Chapter 5), a summary is presented of how this study provides an example for utilizing molecular techniques and evolutionary genetics during biopesticide development. The specific issues include isolate characterization for registration, patent protection, product quality control, evaluation of field efficacy for released isolates and monitoring the fate of introduced isolates in the environment as well as evolutionary genetic analysis for selecting potentially effective isolates.

CHAPTER 2

DNA POLYMORPHISMS IN *BEAUVERIA BASSIANA*: RFLP PROBE DEVELOPMENT AND SEQUENCE ANALYSIS OF THE ITS AND 5.8s rRNA GENE REGION

Introduction

Beauveria bassiana is an entomopathogenic fungus that is a naturally occurring pathogen of many insect species but also is used widely as a biological control agent (Feng et al. 1994). In the United States, *B. bassiana* was experimentally developed for use against Lygus bugs by Nutrilite Products Inc. in the 1960s (Dunn and Mechalas 1963; Ignoffo et al. 1979), and Colorado potato beetles by Abbott Laboratories in the 1980s (Campbell et al. 1985; Hajek et al. 1987; Anderson and Roberts 1983; Anderson et al. 1983, 1988, 1989). In the early 1990s, a *B. bassiana* isolate was developed and registered as the commercial product Naturalis-L ® for controlling boll weevils and whiteflies (Wright and Chandler 1991, 1992a, 1993; Wright 1993). Also, Mycotech registered another *B. bassiana* isolate as the commercial product Mycotrol GHA ®. Mycotrol GHA is effective against whiteflies, thrips, and aphids (Wraight et al. 1996, 1998). Since 1987, isolate 447 (Bb 447) of *B. bassiana* obtained from *Solenopsis invicta* in Brazil has been evaluated in the U.S. as a microbial control agent against fire ants such as *S. invicta* and *S. xyloni* (Stimac et al. 1987, 1993a, 1993b; Oi et al. 1994).

Because different isolates of *B. bassiana* are morphologically indistinguishable, it is necessary to genetically characterize Bb 447 in order to differentiate it from other isolates. In general, defining isolates based on DNA markers will help to establish the genetic identity for particular isolates to meet patenting and registration requirements and to evaluate the efficacy of isolates applied as microbial control agents.

Over the last ten years, a range of molecular techniques has been used to investigate genetic variation in *B. bassiana*. Protein polymorphism studies overcame some of the deficiencies of morphological markers. Isolates of *B. bassiana* could be differentiated based on isoenzyme profiles (Riba et al. 1986; Poprawski et al. 1988; Mugnai et al. 1989; Bridge et al. 1990; St. Leger et al. 1992). However, protein studies have drawbacks. For example, the production of some isozymes may be correlated with particular metabolic events, which may cause inconsistent results. Also, the resolution may be less than that required for the characterization of a specific isolate (Bridge et al. 1990).

DNA-based techniques to study genetic variation in *B. bassiana* have included sequence and restriction analysis of the ribosomal internal transcribed spacer (ITS) and 5.8*s* rRNA gene region (Shih et al. 1995; Glare and Inwood 1998), RAPD-PCR (Bidochka et al. 1994; Maurer et al. 1997a, Glare and Inwood 1998; Castrillo and Brooks 1998; Berretta etal. 1998), pulsed-field gel electrophoresis (PFGE) (Viaud et al. 1996), and restriction fragment length polymorphisms (RFLPs) with single- or low copy homologous DNA probes (Kosir et al. 1991; Hegedus and Khachatourians 1993a; Couteaudier and Viaud 1997) and heterologous DNA probes (Kosir et al. 1991; Maurer et al. 1997a).

RFLP analysis may target two types of DNA: that from specific regions or total genomic DNA. Ribosomal DNA is one of the best-characterized genomic regions available for DNA polymorphism studies in fungi. PCR primers based on the highly conserved rRNA genes have been designed to amplify the polymorphic ITS region (White et al. 1990). RFLP analysis of the ITS region can provide a useful tool for identifying different species or different isolates within the same species (Bruns et al. 1991; Hibbett 1992; Bulat and Mironenko 1996).

As mitochondrial DNA (mtDNA) has a small size, is easy to purify and has a high copy number in a genome for easily visualizing restriction enzyme patterns, and therefore mtDNA has been extensively studied as a specific region for identifying DNA polymorphisms (Bruns et al. 1991). DNA polymorphisms have been successfully revealed among isolates of some fungal species such as *Cryphonectria parasitica* (Milgroom and Lipari 1993), *Fusarium oxysporum* (Kistler et al. 1987; Appel and Gordon 1994), *Cryptococcus neoformans* (Varma and Kwong-Chung 1989), *Armillaria* spp. (Smith and Anderson 1989), *Laccaria* spp. (Grdes et al. 1991) and *Phytophthora megasperma* (Forster et al. 1989). In contrast, no or few DNA polymorphisms were found among isolates of other species, including *Cochliobolus heterostrophus* (Garber and Yoder 1984), *Ophiostoma ulmi* (Jeng et al. 1991), *Ceratocystis fagacearum* (Kurdyla et al. 1995), and *Heterobasidion annosum* (Wingfield et al. 1996). RFLPs of mtDNA can be detected by utilizing homologous or heterologous mtDNA as a probe, which is hybridized to enzyme-digested total DNA of the organism.

RFLP analysis of anonymous genomic loci has also been useful to detect DNA polymorphisms among different isolates in the same species by using homologous or heterologous DNA probes (Bruns et al. 1991; Kohn 1992). Homologous probes may either hybridize to single, low copy or multiple loci in the genome. Among those, moderately repetitive and dispersed DNAs often are hypervariable among isolates of the same fungal species and therefore can be used for DNA fingerprinting (Rosewich and McDonald 1994). Repetitive DNA probes have been developed and used in many plant pathogenic fungi for identification, phylogenetic analysis and population genetic studies, including *Magnaporthe grisea* (Hamer et al. 1989), *F. oxysporum* (Kistler et al. 1991 and Mouyna et al. 1996), *Sclerotinia sclerotiorum* (Kohn et al. 1991), *Mycosphaerella graminicola* (McDonald and Martinez 1991), *C. parasitica* (Milgroom et al. 1992), *Phytophthora infestans* (Goodwin et al. 1992a), *Rhizoctonia solani* (O'Brien 1994), *Aspergillus flavus* (McAlpin and Mannarelli 1995) and *Pythium irregulare* (Matthew et al. 1995). Also, repetitive DNA probes have been developed for medically important fungi such as *Candida albicans* (Scherer and Stevens 1988), *C. neoformans* (Spitzer and Spitzer 1992; Varma and Kwong-Chung 1992), and *A. fumigatus* (Girardin et al. 1993).

Multilocus DNA probes for fingerprinting also can be developed by using telomeric sequences. DNA polymorphisms were revealed by using the telomeric repeated DNA sequences $(TTAGGG)_{18}$ for some fungal species, including *B. bassiana* and *Ustilago maydis* (Viaud et al. 1996; Couteaudier and Viaud 1997; Sanchez-Alonso et al. 1996).

In this study, to genetically characterize *B. bassiana* isolates, particularly isolate Bb 447 and commercial product isolates, sequence analyses of the ITS and 5.8*s* rRNA gene region from isolate Bb 447 and 14 other *B. bassiana* isolates were conducted. In addition, restriction analysis among the ITS and 5.8*s* rRNA gene region was done for nine additional isolates. Secondly, a genomic library of isolate Bb 447 was established to identify polymorphic DNA probes, particularly moderately repetitive sequences. Finally, heterologous telomeric- and mitochondrial probes were evaluated in order to identify some distinct molecular characteristics of *B. bassiana* isolates.

Materials and Methods

Fungal Cultures and *B. bassiana* Isolates

Isolates of *B. bassiana* were maintained in Sabouraud dextrose agar + 0.5% yeast extract (SDAY) plates. After spores formed on these plates, 500 µl of a suspension of 10^7 to 10^8 spores/ml were used to inoculate 100 ml of PD (1% peptone, 2% dextrose) broth in 250-ml Erlenmeyer flasks. The cultures were grown at 25-27°C for 4 days at 170 rpm and harvested into 15-ml polypropylene tubes. *B. bassiana* isolates collected from different *Solenopsis* species with different geographical origins and isolates from commercial products were chosen for sequence analysis of ITS and 5.8*s* rRNA gene region and RFLP probe evaluation (Table 2-1).

DNA Extraction

Frozen tissue of *B. bassiana* was lyophilized for at least two days and then ground with a glass rod. To each tissue tube, 7 ml of DNA extraction buffer were added. The DNA extraction buffer was prepared by a 1: 1: 0.4 solution of buffer A (0.2 M Tris, pH 7.5; 100 mM EDTA, pH 8; 0.2 mM CTAB), buffer B (6.377 % Sorbitol, 1.21 % Trisma base, 0.168 % EDTA, pH 7.5) and Sarkosyl (5 % N-laurylsarkosine). After addition of the DNA extraction buffer, the tubes were incubated in a 65^0C-water bath for 1 hour. After incubation, 1 volume chloroform:octanol (24:1) was added to the tubes. The well-mixed liquid was centrifuged at 15,000 rpm for 20 minutes at 4^0C,

Table 2-1. *Beauveria bassiana* isolates evaluated for DNA polymorphisms based on sequence analysis of ITS and 5.8*s* rRNA gene region or RFLP probes

Isolates	Host or substrate	Origin	ITS [1]	RFLP probes
Bb 446	*Solenopsis invicta*	Cuiaba, MT, Brazil	*	
Bb 447	*Solenopsis invicta*	Cuiaba, MT, Brazil	*	*
Bb 542	*Solenopsis invicta*	Cuiaba, MT, Brazil	*	
Bb 608	Ant nest soil	Cuiaba, MT, Brazil	*	
Bb 614	*Solenopsis saevissima*	Lavras, MG, Brazil	*	*
Bb 883	*Solenopsis quinquecuspis*	Punta del Este, Uruguay		*
FP12C1	*Solenopsis invicta*	Gainesville, Florida	*	
Bb $9H_{11}$	*Solenopsis xyloni*	Bakersfield, California	*	
5715	*Scapteriscus vicinus*	Florida	*	
GHA[2]	*Melanoplus sanguinipes*	Montana	*	*
Naturalis[2]	*Anthonomus grandis grandis*	Rio Grande Valley, Texas	*	*

[1] Sequence analysis of ITS and 5.8*s* rRNA gene region.
[2] Commercial product isolates.

and the upper aqueous phase was transferred to fresh tubes. To each tube, 200 µl of CTAB (10% CTAB, 0.7 M NaCl) and 2 ml of CTAB precipitation buffer (1% CTAB, 50 mM Tris pH 8, 10 mM EDTA pH 8) were added. After being inverted 15 times, the tubes were incubated at room temperature for 20 minutes. The DNA precipitate was collected by centrifugation (12,000 rpm, 20 min, 21^0C). The DNA pellet was resuspended in 7 ml NaCl-TE buffer (1 M NaCl, 10 mM Tris and 1 mM EDTA, pH 8). After resuspension, 1 volume chloroform:octanol (24:1) was added and mixed well. The well-mixed liquid was centrifuged at 15,000 rpm for 20 minutes at 4^0C and the upper aqueous phase was transferred to fresh tubes. One hundred microliters of Proteinase K (20 mg/ml) were added to each tube and incubated at 37 ^{0}C for 1 hour. The DNA was precipitated with 1 volume of isopropanol at room temperature for 10 minutes. After centrifugation (12,000 rpm, 20 minutes, 21^0C), the DNA pellet was resuspended in 400 µl TE buffer. Ten microliters of RNase A were added to each tube. After incubation at 37 ^{0}C for 30 minutes, 2 volumes of 95 % EtOH and 0.5 volume of NH_4OAc were added and the DNA was allowed to precipitate for 20 minutes. The DNA pellet was collected after centrifugation (15,000, 20 min, room temperature). After the ethanol had volatilized, the DNA pellet was resuspended in TE.

PCR-amplification, Sequence and Restriction Analysis of the ITS and 5.8*s* rRNA Gene Region

The primer pair used to amplify the ITS and 5.8*s* rRNA gene region (ITS1/ITS4) has been described by White et al. (1990) and has the following sequences: ITS1: TCCGTAGGTGAACCTGCGG; ITS4: TCCTCCGCTTATTGATATGC. PCR reactions were performed in a 25 µl volume with the following components: 25 mM

$MgCl_2$; 10 mM Tris-HCl, pH 8.3; 10 mM of each of the four dNTPs, 100 ng genomic DNA; 10 ng primer; 0.5 µl *Taq* DNA polymerase (Boehringer Mannheim, Indianapolis, IN 46250). The samples were overlaid with 90 µl mineral oil. Amplification of the ITS and 5.8*s* rRNA gene region was performed in a RoboCycler 40 Temperature Cycler (Stratagene Cloning Systems, La Jolla, CA 92037) programmed for 97°C for 5 min, cooled to 25°C, then brought to 94°C for 3 minutes. This was followed by 45 cycles of 94°C for 1 min, 37°C for 1.5 min, and 72°C for 2 min. Amplification products were separated by electrophoresis with 0.7% agarose gels. The ethidium-bromide stained gel was photographed under UV light. In order to sequence the ITS and 5.8*s* rRNA gene region, PCR products of Bb 447 were purified by the QIAquick PCR Purification Kit and directly sequenced by the dideoxynucleotide chain termination method (DNA sequence Lab, Institute of Food and Agricultural Science, University of Florida). Alignment analysis of DNA sequences was performed with Sequencher Version 3.0 (GeneCodes Corporation, Ann Arbor, MI 48106) for the ITS and 5.8*s* rRNA gene regions of Bb 447 and 14 other *B. bassiana* isolates, which were obtained from GenBank. Also, sequence similarities between Bb 447 and these 14 other isolates were analyzed with Bestfit program in Wisconsin Sequence Analysis Package Version 8.0 (Genetic Computer Group, University Research Park, Madison, WI 53711).

The ITS and 5.8*s* rRNA gene region of nine isolates associated with *Solenopsis* spp. and other insect hosts (Table 2-1) was amplified and digested with various restriction enzymes. Digested PCR products were detected after separation by Nusieve GTG: LE Agrose (%) (3:1) gels. In addition, the existence of fragments with small sizes was confirmed by hybridizing the PCR product of total ITS and 5.8*s* rRNA gene region of Bb 447 to Southern blots.

Constructing and Screening of a Genomic Library

Genomic DNA of the Bb 447 was digested to completion with *Eco* RI, phenol/chloroform extracted and precipitated with ethanol. The precipitate was then washed twice with 70% ethanol. The DNA was air-dried and suspended in TE. The digested DNA was ligated overnight at 15^0C to *Eco* RI-digested plasmid vector pBluescript KS (+), according to the instructions of the T4 DNA ligase manufacturer (GibcoBRL, Rockville, MD 20849). The ligated DNA was used to transform host cells (*E. coli* DH5α MCR). The cell/DNA mixture was incubated in LB media with continuous shaking at 37^0C for 1 h. Aliquots were distributed on LB-agar plates supplemented with carbencillin (75 μg/ml), and X-GAL (2%) for colorometric selection of transformants. The small-scale alkaline-lysis method of Maniatis et al. (1982) was used to lyse the bacterial cells and to isolate the plasmid DNA. Labeled probes were prepared by nick translating 100 ng of target DNA with ^{32}P-dCTP.

In order to identify repetitive DNA sequences, recombinant clones from a genomic library of Bb 447 were hybridized to labelled genomic DNA of Bb 447. As the clones that gave strong hybridization signals were assumed to contain repetitive DNA inserts, only these clones were selected for further screening. Also, only non-rDNA, non-mtDNA repetitive nuclear DNA sequences were considered good candidates to provide polymorphic probes. To identify and to exclude recombinant clones containing rDNA or mtDNA, all clones with strong hybridization signals were hybridized to the PCR product of the ITS and 5.8*s* rRNA gene region from Bb 447, and to the ribosomal DNA unit of *F. oxysporum* ((Kistler et al. 1987) and to the mtDNA of *F. oxysporum* f. sp. *conglutinans*. Finally, the remaining clones were hybridized to Southern blots of *Eco* RI-digested genomic DNA of Bb 447 to identify those clones that displayed multiple banding patterns.

Enzyme Digestion, Electrophoresis, Southern Blotting and Probe Choice

Five micrograms of purified DNA were digested individually with the restriction enzymes *Eco* RI, *Hin*d III, and *Pst* I. The digested DNA was size-separated by electrophoresis through 0.8 % agarose-TBE (40 mM Tris, 20 mM Boric acid, 20 mM EDTA) gels. Electrophoretically separated DNA samples were denatured and transferred to a Zeta Probe membrane (BIO-RAD Laboratories, Hercules, CA 94547) with the method developed by Southern (1975). The membrane was baked at 80^0C for 30 minutes. After prehybridization for 60 minutes in the prehybridization solution (0.25 M NaH_2PO_4, pH 7.2 and 7% SDS), the heat-denatured, labelled probe was added to the hybridization solution, which was the same as the prehybridization solution, and incubated in a hybridization oven with rolling bottles at 65^0 overnight. The membrane was washed twice at 65^0C with wash solution # 1 (20 mM Na_2HPO_4, 5 % SDS) and twice with wash solution # 2 (20m M Na_2HPO_4, 1 % SDS). Each wash cycle lasted 15 minutes. The membrane was then wrapped in plastic wrap and exposed to X-ray film at -80^0C for one day.

To distinguish Bb 447 and other isolates from fire ants from two commercial products of *B. bassiana* (Table 2-1), the cloned repetitive sequences derived from the genomic library were used as homologous DNA probes. Also, a pUC12 plasmid clone containing 18 repeats of the telomeric sequence TTAGGG from *F. oxysporum* and mitochondrial DNA from *F. oxysporum* f. sp. *conglutinans* were used as heterologous DNA probes (Powell and Kistler 1990; Kistler and Benny 1989).

Results

Sequencing and Restriction Analysis of the ITS and 5.8*s* rRNA Gene Region

When the sequenced PCR product of the ITS and 5.8*s* rRNA gene region from Bb 447 was aligned with sequences of 14 other *B. bassiana* isolates using Bestfit program, sequence similarities between Bb 447 and other isolates were varied, from 91.2% (Bb 447 and Z54111) to 99.6 % (Bb 447 and Bb18092). Sequence heterogeneity of the ITS and 5.8*s* rRNA gene regions among these 15 isolates is shown in Fig. 2-1. No variation was found in either the sizes of the ITS and 5.8*s* rRNA gene region or banding patterns when the ITS and 5.8*s* rRNA gene regions from nine isolates associated with *Solenopsis* spp. and other insect hosts was amplified, digested with *Alu* I, *Hae* III or *Sau* 3AI and electrophoresed (Table 2-2).

Isolation and Evaluation of Recombinant Clones

Out of 192 recombinant clones from the genomic library of Bb 447, 36 hybridized strongly to labeled genomic DNA from Bb 447. Out of these 36 clones, ten were identified as either mitochondrial DNA (4 clones) or ribosomal DNA (6 clones). Finally, when the 26 remaining clones were further hybridized to Southern blots of *Eco* RI-digested genomic DNA of isolate Bb 447, three gave multiple bands, indicating that the inserts contained non-ribosomal repetitive DNA sequences. The remaining 23 clones resulted in single bands and therefore were confirmed as single-

```
          1                                                    50
U18951    A.ACCCTTCT GTG.AACCTA CCTATCGTTG CGTTCGGCGG A.CTCACCC.
U18093    ACACCCTTCT GTG.AACCTA CCTATCGTTG C.TTCGGCGG A.CTCGCCCC
U18092    A.ACCCTTCT GTG.AACCTA CCTATCGTTG C.TTCGGCGG AGCTCGCCCC
U18948    A.ACCCTTCT GTG.AACCTA CCTATCGTTG C.TTCGGCGG A.CTCGCCCC
U18949    A.ACCCTTCT GTGGAACCTA CCTATCGTTG C.TTCGGCGG A.CTCGCCCC
U18950    A.ACCCTTCT TTG.AACCTA CCTATCGTTG C.TTCGGCGG A.CTCGCCCC
U18946    A.ACCCTTCT GTG.AACCTA CCTATCGTTG C.TTCGGCGG A.CTCGCCCC
U18952    A.ACCCTTCT GTG.AACCTA CCTATCGTTG C.TTCGGCGG A.CTCGCCCC
U18787    A.ACCCTTCT GTG.AACCTA CCTATCGTTG C.TTCGGCGG A.CTCGCCCC
U18947    A.ACCCTTCT GTG.AACCTA CCTATCGTTG C.TTCGGCGG A.CTCGCCCC
U18786    A.ACCCTTCT GTG.AACCTA CCTATCGTTG C.TTCGGCGG A.CTCGCCCC
U18788    A.ACCCTTAT GT..AACCTA CCTATTGTTG C.TTCGGCGG A.CTCGCCCC
Bb 447    ..ACCCTTCT GTG.AACCTA CCTATCGTTG C.TTCGGCGG A.CTCGCCCC
U18953    A.ACCCTTCA ATG.AACCTA CCTATCGTTG C.TTCGGCGG G.CTCGCCCC
Z54111    A.ACCCTTCA ATG.AACCTA CCTATCGTTG C.TTCGGCGG G.CTCGCCCC

          51                                                  100
U18951    AGC..CCGGA CGCGGGCTGG ..CC.GC..C ..G...GGGA CCCT..AAAC
U18093    AGC..CCGGA CGCGGACTGG A.CCAGCGGC CCGCC.GGGG CCCT.CAAAC
U18092    AGC..CCGGA CGCGGACTGG A.CCAGCGGC CCGCCTGGGG ACCT.CAAAC
U18948    AGC..CCGGA CGCGGACTGG A.CCAGCGGC CCGCC.GGGG CCCT.CAAAC
U18949    AGC..CCGGA CGCGGACTGG AGCCAGCGGC CCGCC.GGGG CCCT.CAAAC
U18950    AGC..CCGGA CGCGGACTGG A.CCAGCGGC CCGCC.GGGG CCCT.CAAAC
U18946    AGC..CCGGA CGCGGACTGG A.CCAGCGGC CCGCC.GGGG CCCT.CAAAC
U18952    AGC..CCGGA CGCGGACTGG A.CCAGCGGC CCGCC.GGGG CCCT.CAAAC
U18787    AGC..CCGGA CGCGGACTGG A.CCAGCGGC CCGCC.GGGG CCCT.CAAAC
U18947    AGC..CCGGG CGCGGACTGG G.CCAGCGGC CCGCC.GGGG ACCT..AAAC
U18786    AGC..CCGGA CGCGGACTGG A.CCAGCGGC CCGCC.GGGG ACCT.CAAAC
U18788    AGC..C.GGA CGCGGACTGG A.CCAGCGGC C.GTC.GGGG ACCT.CAAAC
Bb 447    AGC..CCGGA CGCGGACTGG A.CCAGCGGC CCGCC.GGGG ACCT.CAAAC
U18953    AGCGTCCGGA CG.GCCCCGC G.CCGGCCCG CGGCCTGGAC CCAGGCGGCC
Z54111    AGCGTCCGGA CG.GCCCCGC G.CCGGCCCG CGGCCTGGAC CCAGGCGGCC

          101                                                 150
U18951    TCTTGT.ATT AT.C.AG.C. ..ATC..T.A ......CTGA ATACGCCGCA
U18093    TCTTGT.ATT GT...ATTCC ..AGC.ATC. .T.T..CTGA ATAGGCCGCA
U18092    TCCTGT.A.. .TT.....CC ..AGC.ATC. .T.T..CTGA ATACGCCGCA
U18948    TCTTGT.A.. .TTCCAG.C. ..ATC..T.A ......CTGA ATACGCCGCA
U18949    GGCTGT.A.. .TTCCAG.C. ..ATC..T.. .T....CTGA ATACGCCGCA
U18950    TCCTGT.A.. .TTCCAG.C. ..ATC..T.A ......CTAA ATAGGCCGCA
U18946    TCTTGT.A.. .TTCCAG.C. ..ATC..T.A ......CTGA ATACGCCGCA
U18952    TCTTGT.A.. .TTCCAG.C. ..ATC..T.. .T....CTGA ATACGCCGCA
U18787    TCTTGT.A.. .TTCCAG.C. ..ATC..T.A ......CTGA ATACGCCGCA
U18947    TCTTGT.A.. .TTCCAG.C. ..ATC..T.. .T....CTGA ATACGCCGCA
U18786    TCTTGT.A.. .TTCCAG.C. ..ATC..T.. .T....CTGA ATACGCCG.A
U18788    TCTTGT.A.. .TTCCAG.C. ..ATC..T.. .T....CTGA ATACGCCGCA
Bb 447    TCTTGT.A.. .TTCCAG.C. ..ATC..T.. .T....CTGA ATACGCCGCA
U18953    GCCGGAGACC CTTCCAGCCT GTATCCATCA GTCTTTCTGA ATCCGCCGCA
Z54111    GCCGGAGACC CTTCCAGCCT GTATCCATCA GTCTTTCTGA ATCCGCCGCA
```

Fig. 2-1. Sequence alignment of the ITS and 5.8*s* rRNA gene regions for *Beauveria bassiana* isolate 447 (Bb 447) and 14 other isolates obtained from Genbank. (All dots except at both ends of the sequences note deletions.)

```
          151                                                200
U18951    AGGCAAA.AC AAATAAATTA AAA.CTTT.C AA.CAACGG. ATCTC.TTGG
U18093    AGGCACAA.C AAATGAATCA AAA.CTTT.C AA.CAACGG. ATC.TCTTGG
U18092    AGGCAAAAAC AAATGAATCA AAA.CTTT.C AA.CAACGG. ATCGTCTTGG
U18948    AGGCAAAA.C AAATGAATCA AAA.CTTT.C AA.CAACGG. ATCTC.TTGG
U18949    AGGCAAAAAC AAATGAATCA AAA.CTTT.C AA.CAGCGG. ATCTC.TTGG
U18950    AGGAAAA.AC AAATGAATCA AAA.CTTT.C AA.CAACGG. ATCTC.TTGG
U18946    AGGAAAACA. AAATGAATCA AAA.CTTT.C AA.CAACGGG ATC.TCTTGG
U18952    AG.CAAAA.C AAATGAATCA AAA.CTTT.C AA.CAACGG. ATCTC.TTGG
U18787    AGGCAAA.AC AAATGAATCA AAA.CTTT.C AA.CAACGG. ATCTC.TTGG
U18947    AGGCAAA.AC AAATGAATCA AA.GCTTG.C AAGCAAGCGG ATCGC.TTGG
U18786    AG.CAAA.AC AAATGAATCA AAAGCTTTGC AAGCAAGCGG ATCTCCTTGG
U18788    AGGAAAACA. AA.TGAATCA AAA.CTTT.C AA.CAACGG. ATC.TCTTGG
Bb 447    AGGCAAA.AC AAATGAATCA AAA.CTTT.C AA.CAACGG. ATCTC.TTGG
U18953    AGGCAAA..C AAATGAATCA AAA.CTTT.C AA.CAACGG. ATCTC.TTGG
Z54111    AGGCAAA..C AAATGAATCA AAA.CTTT.C AA.CAACGG. ATCTC.TTGG

          201                                                250
U18951    CTCTGGCATC GATGAAGAAC GCAGCGAAA. CGCGATAAGT AATGTGAATT
U18093    CTCTGGCATC GATGAAGAGC GCAGCG.AAA TGCGATAAGT AATGTGAATT
U18092    CTCTGGCATC GATGAAGAAC GCAGCG.AAA TGCGATAAGT AATGTGAATT
U18948    CTCTGGCATC GATGAAGAAC GCAGCGAAAT .GCGATAAGT AATGTGAATT
U18949    CTCTGGCATC GATGAAGAAC GCAGCGAAAT .GCGATAAGT AATGTGAATT
U18950    CTCTGGCATC GATGAAGAAC GCAGCGAAAT .GCGATAAGT AATGTGAATT
U18946    CTCTGGCATC GATGAAGAAC GCAGCGTAAA CGCGATAAGT AATGTGAATT
U18952    CTCTGGCATC GATGAAGAAC GCAGCGAAAC .GCGATAAGT AATGTGAATT
U18787    CTCTGGCATC GATGAAGAAC GCAGCGAAAT .GCGATAAGT AATGTGAATT
U18947    CTCTGG.ATC GATGAAGAGC GCAGCGAAAT .GCGATAAGT AATGTGAATT
U18786    CTCTGGCATC GATGAAGAAC GCAGCGAAAG CGCGATAAGT AATGTGAATT
U18788    CTCTGGCATC GATGAAGAAC GCAGCG.AAA TGCGATAAGT AATGTGAATT
Bb 447    CTCTGGCATC GATGAAGAAC GCAGCGAAAT .GCGATAAGT AATGTGAATT
U18953    TTCTGGCATC GATGAAGAAC GCAGCGAAAG CGCGATAAGT AATGTGAATT
Z54111    TTCTGGCATC GATGAAGAAC GCAGCGAAAG CGCGATAAGT AATGTGAATT

          251                                                300
U18951    GCAGAATCCA .GTGAATC.A TCGAATCTTT GAACGCACAT .TG.CGCC.C
U18093    GCAGAATC.C AGTGAATC.A TCGAATCTTT GAACGCACAT .TTGCGCC.C
U18092    GCAGAATC.C AGTGAATC.A TCGAATCTTT GAACGCACAT .T.GCGCC.C
U18948    GCAGAATCCA .GTGAATC.A TCGAATCTTT GAACGCACAT .TG.CGCC.C
U18949    GCAGAATCCA .GTGAATC.A TCGAATCTTT GAACGCACAT .TG.CGCC.C
U18950    GCAGAATCCA AGTGAATC.A TCGAATCTTT GAACGCACAT .TG.CGCC.C
U18946    GCAGAATCGC AGTGAATCGA TCGAATCTTT GAACGCACAT .T.GCGCC.C
U18952    GCAGAATCCA .GTGAATC.A TCGAATCTTT GAACGCACAT .TGGCGCC.C
U18787    GCAGAATCCA .GTGAATC.A TCGAATCTTT GAACGCACAT .TG.CGCC.C
U18947    GCAGAATCCA .GTGAATC.A TCGAATCTTT GAACGCACAT .TG.CGCC.C
U18786    GCAGAATCCA .GTGAATC.A TCGAATCTTT GAGCGCACAT .TG.CGCCGC
U18788    GCAGAATC.C AGTGAATC.A TCGAATCTTT GAACGCACAT .T.GCGCC..
Bb 447    GCAGAATCCA .GTGAATC.A TCGAATCTTT GAACGCACAT .TG.CGCC.C
U18953    GCAGAATTTA .GTGAATC.A TCGAATCTTT GAACGCACAT .T.GCTGCCC
Z54111    GCAGAATTTA .GTGAATC.A TCGAATCTTT GAACGCACAT ATTGCTGCCC
```

Fig. 2-1. Continued

```
              301                                            350
U18951        GCCAGCATTC TGGCGGGCTA TGCCT..GTT CGAG.CGTCA TTACAACCC.
U18093        GCCAGCATTC TGGCGGGC.A TGCCT..GTT CGAG.CGTCA TTTCAACCC.
U18092        GCCAGCATTC TGGCGGGC.A TGCCT..GTT CGAG.CGTCA TTTCAACCC.
U18948        GCCAGCATTC TGGCGGGC.A TGCCT..GTT CGAG.CGTCA TTTCAACCC.
U18949        GCCAGCATTC TGGCGGGC.A TGCCT..GTT CGAG.CGTCA TTTCAACCC.
U18950        GCCAGCATTC TGGCGGGC.A TGCCT..GTT CGAG.CGTCA TTTCAACCC.
U18946        GCCAGCATTC TGGCGGGC.A TGCCTTTGTT CGAG.CGTCA TTTCAACCC.
U18952        GCCAGCATTC TGGCGGGC.A TGCCT..GTT CGAG.CGTCA TTTCAACCC.
U18787        GCCAGCATTC TGGCGGGC.A TGCCT..GTT CGAGGCGTCA TTTCAACCC.
U18947        GCCAGCATTC TGGCGGGC.A TGCCT..GTT CGAG.CGTCA TTTCAACCC.
U18786        GCCAGCATTC TGGCGGGC.A TGCCT..GTT CGAG.CGTCA TATCAACCCC
U18788        GCCAGCATTC TG.CGGGC.A TGCCTGT.TT CGAG.CGTCA TTTCAACCC.
Bb 447        GCCAGCATTC TGGCGGGC.A TGCCT..GTT CGAG.CGTCA TTTCAACCC.
U18953        GGCAGCATTC TGGCGGGC.A TGCCT..GTT CGAG.CGTCA TTTCAACCC.
Z54111        GGCAGCATTC TGGCGGGC.A TGCCT..GTT CGAG.CGTCA TTTCAACCC.

              351                                            400
U18951        TCGA.CTTCC C.TAGGGAAG .T.CGGCGTT GGGGACCGGC AGCACACCGC
U18093        TCGA.CCTCC CCTTGGGGAG GT.CGGCGTT GGGGACCGGC AGCACACCGC
U18092        TCGA.CCTCC CCTTGGGGAG GT.CGGCGTT GGGGACCGGC AGCACACCGC
U18948        TCGA.CCTCC CCTGGGGGAG GT.CGGCGTT GGGGACCGGC AGCACACCGC
U18949        TCGA.CCTCC CCTTGGGGAG GT.CGGCGTT GGGGACCGGC AGCACACCGC
U18950        TCGA.CCTCC CCTTGGGGAG GT.CGGCGTT GGGGACCGGC AGCACACCGC
U18946        TCGA.CCTCC CCTTGGGGAG GT.CGGCGTT GGGGACCGGC AGCACACCGC
U18952        TCGA.CCTCC CCTTGGGGAG GT.CGGCGTT GGGGACCGGC AGCACACCGC
U18787        TCGA.CCTCC CCTTGGGGAG GT.CGGCGTT GGGGACCGGC AGCACACCGC
U18947        TCGA.CCTCC CCTTGGGGAG GT.CGGCGTT GGGGACCGGC AGCACACCGC
U18786        TCGAACCTCC CCTTGGGGAG GT.CGGCGTT GGGGACCGGC AGCACACCGC
U18788        TCGA.CCTCC CTTTGGGGAA GT.CGGCGTT GGGGACCGGC AGCACACCGC
Bb 447        TCGA.CCTCC CCTGGGGGAG GT.CGGCGTT GGGGACCGGC AGCACACCGC
U18953        TCGA.CGTCC CCT.GGGGAC GTGCGGCCTT GGGGACCGGC AGCCCACCGC
Z54111        TCGA.CGTCC CCT.GGGGAC GTGCGGCCTT GGGGACCGGC AGCCCACCGC

              401                                            450
U18951        CGGCCCTGAA ATGGAGTGGC GGCCCGTCCG CGGCGACCTC TGCGTAGTAA
U18093        CGGCCCTGAA ATGGAGTGGC GGCCCGTCCG CGGCGACCTC TGCGTAGTAA
U18092        CGGCCCTGAA ATGGAGTGGC GGCCCGTCCG CGGCGACCTC TGCGTAGTAA
U18948        CGGCCCTGAA ATGGAGTGGC GGCCCGTCCG CGGCGACCTC TGCGTAGTAA
U18949        CGGCCCTGAA ATGGAGTGGC GGCCCGTCCG CGGCGACCTC TGCGTAGTAA
U18950        CGGCCCTGAA ATGGAGTGGC GGCCCGTCCG CGGCGACCTC TGCGTAGTAA
U18946        CGGCCCTGAA ATGGAGTGGC GGCCCGTCCG CGGCGACCTC TGCGCAGTAA
U18952        CGGCCCTGAA ATGGAGTGGC GGCCCGTCCG CGGCGACCTC TGCGCAGTAA
U18787        CGGCCCTGAA ATGGAGTGGC GGCCCGTCCG CGGCGACCTC TGCGTAGTAA
U18947        CGGCCCTGAA ATGGAGTGGC GGCCCGTCCG CGGCGACCTC TGCGTAGTAA
U18786        CGGCCCTGAA ATGGAGTGGC GGCCCGTCCG CGGCGACCTC TGCGCAGTAA
U18788        CGGCCCTGAA ATGGAGTGGC GGCCCGTCCG CGGCGACCTC TGCGTAGTAA
Bb 447        CGGCCCTGAA ATGGAGTGGC GGCCCGTCCG CGGCGACCTC TGCGTAGTAA
U18953        CGGCCCTGAA ATCGAGTGGC GGCCCGTCCG CGGCGACCTC TGCGCAGTAC
Z54111        CGGCCCTGAA ATCGAGTGGC GGCCCGTCCG CGGCGACCTC TGCGCAGTAC
```

Fig. 2-1. Continued

```
          451                                              500
U18951    ACCAACTCGC ACC.GGAACC CCGACGTGGC CATGCCGTAA AAC.ACCCA.
U18093    TACAGCTCGC ACC.GGAACC CCGACGCGGC CACGCCGTAA AAC.ACCCAA
U18092    TACAGCTCGC ACC.GGAACC CCGACGCGGC CACGCCGTAA AAC.ACCCAA
U18948    TACAGCTCGC AGCCGGAACC CCGACGCGGC CACGCCGTAA AAC.ACCCAG
U18949    TACAGCTCGC ACCGGGAACC CCGACGCGGC CACGCCGTAA AAC.ACCCAA
U18950    TACAGCTCGC ACC.GGAACC CCGACGCGGC CACGCCGTAA AAC.ACCCAA
U18946    TACAGCTCGC ACC.GGGACC CCGACGCGGC CACGC.GTAA AAC.ACCCAA
U18952    TACAGCTCGC ACC.GGAACC CCGACGCGGC CACGCCGTAA AAC.ACCCAA
U18787    TACAGCTCGC ACC.GGAACC CCGACGCGGC CACGCCGTAA AACCACCCAA
U18947    TACAGCTCGC ACC.GGAACC CCGACGCGGC CACGCCGTAA AAC.ACCCAA
U18786    TACAGCTCGC ACC.GGGACC CCGACGCGGC CACGCCGTAA AAC.ACCCAA
U18788    TCCAACTCGC ACC.GGAACC CCGACGCGGC CACGCCGTAA AAC.ACCCAA
Bb 447    TACAGCTCGC ACC.GGAACC CCGACGCGGC CACGCCGTAA AAC.ACCCAA
U18953    TCCAGCTCGC ACC.GGGAAC CCGACGCGGC CACGCT.TAA AAC.ACCCAA
Z54111    TCCAGCTCGC ACC.GGGAAC CCGACGCGGC CACGCT.TAA AAC.ACCCAA

          501                                              550
U18951    CTTCTAAACG TTGACCTCGA ATCAGGTAGG .CTACCCTTC TGAACTTAAG
U18093    CTTCTGAACG TTGACCTCGA ATCAGGTAGG .CTACCCG.C TGAACTTAAG
U18092    CTTCTGAACG TTGACCTCGA ATCAGGTAGG .CTACCCG.C TGAACTTAAG
U18948    CTTCTGATCG TTGACCTCGA ATCAGGTAGG .CTACCCG.C TGAACTTAAG
U18949    CTTCTGAACG TTGACCTCGA ATCAGGTAGG ACTACCCG.C TGAACTTAAG
U18950    CTTCTGAACG TTGACCTCGA ATCAGGTAGG ACTACCCG.C TGAACTTAAG
U18946    CTTCTGAACG TTGACCTCGA ATCAGGTAGG .CTACCCG.C TGAACTTAAG
U18952    CTTCTGAACG TTGACCTCGA ATCAGGTAGG .CTACCCG.C TGATCTTAAG
U18787    CTTCTGAACG TTGACCTCGA ATCAGGTAGG ACTACCCG.C TGAACTTAAG
U18947    CTTCTGAACG TTGACCTCGA ATCAGGTAGG ACTACCCT.C TGAACTTAAG
U18786    CTTCTGAACG TTGACCTCGA ATCAGGTAGG ACTACCCG.C TGAACTTAAG
U18788    CTTCTGAACG TTGACCTCGA ATCAGGTAGG ACTACCCG.C TGAACTTAAG
Bb 447    CTTCTGAACG TTGACCTCGA ATCAGGTAGG ACTACCCG.C TGAACTTAAG
U18953    CT.CTGAACG TTGACCTCGG AT.AGGTAGG ACTACCCTTC TGAACTTAAG
Z54111    CT.CTGAACG TTGACCTCGG AT.AGGTAGG ACTACCCTTC TGAACTTAAG

          551    560
U18951    CATATCAAT
U18093    CATATCAAT
U18092    CATATCAAT
U18948    CATATCAT.
U18949    CATATCAAT
U18950    CATATCAAT
U18946    CATATCAAT
U18952    CATATCAAT
U18787    CATATCAAT
U18947    CATATCAAT
U18786    CATATCAAT
U18788    CATATCAAT
Bb 447    CATATCAAT
U18953    CATATCAAT
Z54111    CATAT....
```

Fig. 2-1. Continued

Table 2-2. Restriction enzyme analysis of the ITS and 5.8*s* rRNA gene region

Isolates	**Region size**	***Alu* I**	***Hae* III**	***Sau* 3AI**
Bb 446	517*	--**	286, 85, 71, 56, 19	--
Bb 447	517	409, 108	286, 85, 71, 56, 19	360, 157
Bb 542	517	--	--	360, 157
Bb 608	517	409, 108	286, 85, 71, 56, 19	--
Bb 614	517	409, 108	286, 85, 71, 56, 19	--
FP12C1	517	--	286, 85, 71, 56, 19	360, 157
Bb $9H_{11}$	517	--	286, 85, 71, 56, 19	360, 157
5715	517	--	286, 85, 71, 56, 19	360, 157
GHA	517	409, 108	--	360, 157
Naturalis	517	409, 108	286, 85, 71, 56, 19	--

* Fragment sizes are in base pairs.

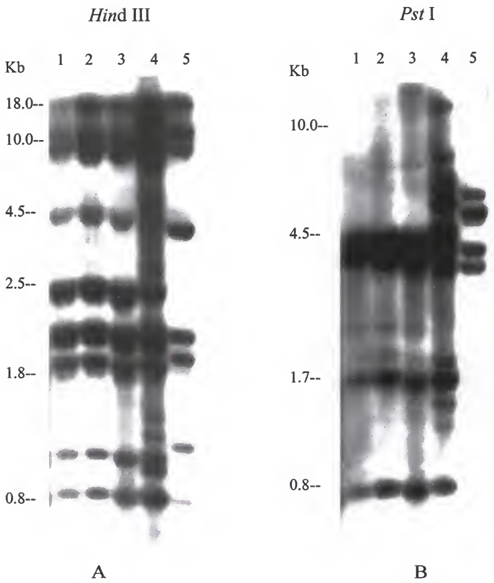
** Isolate-enzyme combination was not tested.

copy genomic sequences. The three multi-copy clones were designated as probe 133 (8 kb), probe 205 (6.0 kb) and probe 520 (1.3 kb and 1.5 kb).

DNA Polymorphisms by Using Homologous DNA Probes

When hybridized to the repetitive probe 133, DNA from all isolates derived from fire ants produced identical banding patterns. The banding pattern consisted of ten fragments for *Hin*d III-digested DNA and five fragments for *Pst* I-digested DNA ranging in size from 0.8 kb to 18.0 kb (Fig. 2-2A and B). For *Hin*d III-digested DNA of the commercial product isolate Naturalis, most bands were shared with those observed in isolates from fire ants (Fig.2-2A). However, two fragments (2.5 kb and 4.5 kb) that were shared among isolates from fire ants were absent in Naturalis. Conversely, Naturalis displayed one extra fragment (4.2 kb). The banding pattern of *Pst* I-digested DNA of Naturalis also was distinguishable from those produced by isolates from fire ants by the presence of 4.8 kb and 5.5 kb fragments and the absence of 0.8 kb and 1.7 kb fragments (Fig. 2-2B). In comparison to all other isolates, isolate GHA generated larger size fragments for both *Hind* III- and *Pst* I-digested DNA and displayed unique patterns (Fig. 2A and B).

Similarly, when probe 205 was hybridized to *Hin*d III- and *Pst* I-digested genomic DNA, three different multiple-copy banding patterns were generated among isolates from fire ants, GHA and Naturalis (Fig. 2-3). Three other clones, probes 520, 501, and 805 were hybridized to *Hin*d III- and *Pst* I-digested genomic DNA. Among the six enzyme-probe combinations, only probe 501 in conjunction with *Hin*d III-digested DNA could distinguish between GHA, Naturalis and isolates from fire ants (including Bb 447) (Table 2-3).



*Hin*d III

Pst I

Fig 2-2. DNA fingerprinting profile of *Beauveria bassiana* isolates by using probe 133. Lanes 1-5: Bb 614, Bb 883, Bb 447, GHA and Naturalis. Genomic DNA was digested with *Hin*d III (A) and *Pst* I (B).

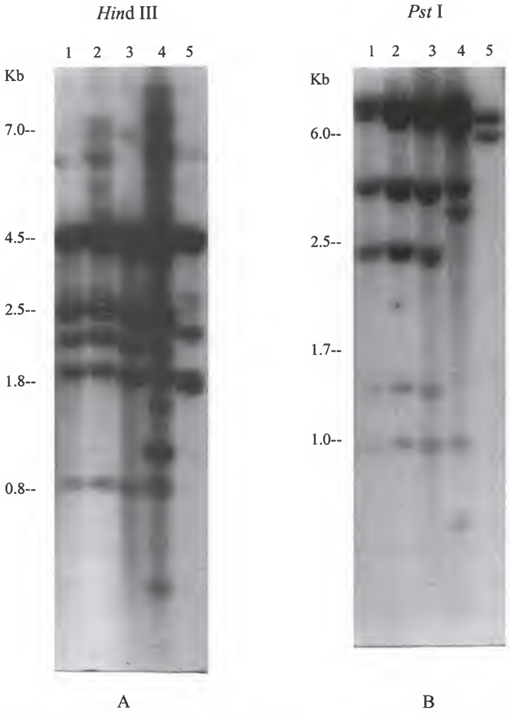


Fig 2-3. DNA fingerprinting profile of *Beauveria bassiana* isolates by using probe 205. Lanes 1-5: Bb 614, Bb 883, Bb 447, GHA and Naturalis. Genomic DNA was digested with *Hind* III (A) and *Pst* I (B).

Table 2-3. RFLP banding patterns among isolates from fire ants, GHA and Naturalis by single and low copy homologous RFLP probes

	*Hin*d III			*Pst* I		
Probes	Ants	GHA	Nat.	Ants	GHA	Nat.
501	14.0*	--	--	--	--	7.0
	--	8.0	8.0	3.8	3.8	--
	6.2	6.2	--	1.8	1.8	1.8
	1.0	1.0	1.0	1.1	1.1	1.1
520	18.0	18.0	18.0	1.0	1.0	--
	12.0	12.0	12.0	--	--	3.8
	5.9	5.9	5.9	--	--	3.0
	2.2	2.2	2.2			
	2.0	2.0	2.0			
	1.4	--	--			
805	--	4.5	--	16.0	16.0	16.0
	--	4.0	--			
	--	2.8	--			
	2.0	2.0	2.0			
	1.8	1.8	1.8			

*Fragment sizes are in kb.

DNA Polymorphisms by Using Heterologous DNA Probes

When the telomeric sequence $(TTAGGG)_{18}$ was hybridized to *Eco* RI, *Hin*d III, *Pst* I, and *Xba* I digested genomic DNA, *Hin*d III-digested DNA produced the most restriction fragments (12-15 fragments) ranging from 0.2 to 16 kb for all isolates (Fig. 2-4A). Specifically, two out of three isolates associated with fire ants (Bb 447 and Bb 883) showed identical DNA banding patterns. The third isolate, Bb 614 shared most fragments with the other two isolates, except for the lack of a 2.2 kb fragment and the presence of a 4.0 kb fragment. Conversely, the two commercial isolates (GHA and Naturalis) showed unique DNA banding patterns with the probe-enzyme combination telomeric probe/*Hin*d III. For *Pst* I-digested DNA, Bb 614 shared the same fragments with the other two isolates from fire ants except for the lack of a 1.5 kb fragment (Fig. 2-4B). Again, GHA, Naturalis and isolates from fire ants displayed very different RFLP patterns.

Based on results from mitochondrial DNA from *F. oxysporum* as a probe, digested genomic DNA of all isolates shared major fragments: 20.0 kb and 1.8 kb for *Eco* RI-digested DNA; 1 kb, 1.4 kb, and 2.5 kb for *Hin*d III-digested DNA (Fig. 2-5). More specifically, all isolates associated with fire ants (Bb 447, Bb 614 and Bb 883) showed identical DNA banding patterns with both enzymes. For *Hin*d III-digested DNA, the only difference between Naturalis and isolates from fire ants was the presence of a 7 kb fragment in Naturalis instead of a 7.2 kb fragment, which was present among isolates from fire ants. In general, isolate GHA exhibited more fragments and showed unique banding patterns for both *Eco*R I- and *Hin*d III- digested DNA.

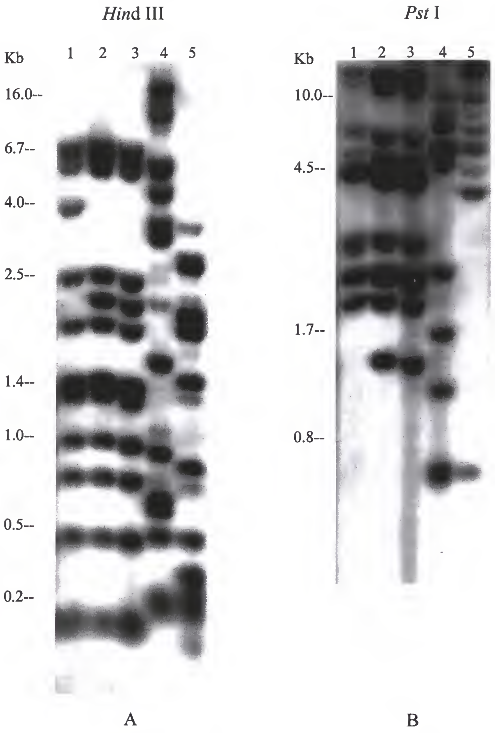


Fig 2-4. DNA fingerprinting profile of *Beauveria bassiana* isolates by using a telomeric probe from *Fusarium oxysporum.* Lanes 1-5:Bb 614, Bb 883, Bb 447, GHA and Naturalis. Genomic DNA was digested with *Hin*d III (A) and *Pst* I (B).

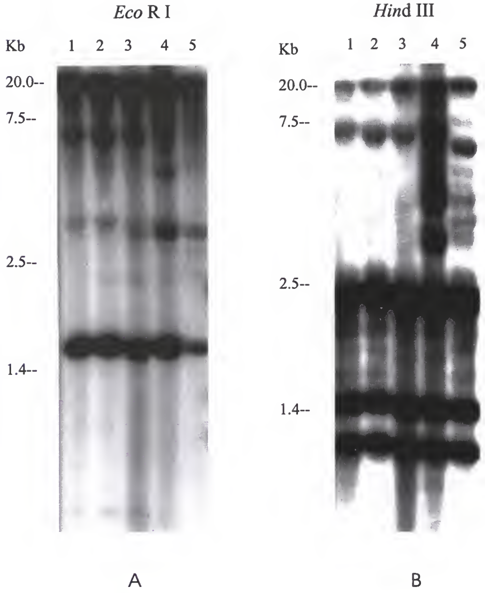


Fig 2-5. DNA banding patterns of *Beauveria bassiana* isolates by using a mitochondrial probe from *Fusarium oxyporum.* Lanes 1-5:Bb 614, Bb 883, Bb 447, GHA and Naturalis. Genomic DNA was digested with *Eco* RI (A) and *Hind* III (B).

Discussion

There are many DNA-based techniques to differentiate between isolates within a fungal species (Bruns et al. 1991; Kohn, 1992). Compared to hybridization-based methods, PCR-based methods are less expensive and are especially useful to target specific DNA regions. For example, restriction analysis of PCR products from the ITS and 5.8*s* rRNA gene region among 28 isolates of *B. brongniartii* separated the isolates into several distinct groups (Neuveglise et al. 1994). Also, this approach was successful in subgrouping morphologically indistinguishable isolates in other fungal species (Henrion et al. 1994; Ward and Akrofi 1994).

This study identified DNA sequence heterogeneity in the ITS and 5.8*s* rRNA gene regions among Bb 447 and 14 other *B. bassiana* isolates. Due to cost and time considerations, we chose restriction analysis rather than sequence analysis for PCR products of the ITS and 5.8*s* rRNA gene region to analyze an additional nine isolates. No variation was found among the nine isolates after the PCR products of the ITS and 5.8*s* rRNA gene region were digested with *Alu* I, *Hae* III or *Sau* 3AI. Similarly, a recent study did not identify any restriction sites for *Bgl* II, *Dra* I or *Kpn* I in the ITS and 5.8*s* rRNA gene region of 11 *B. bassiana* isolates. In addition, no differences were observed in banding patterns in digests with *Alu* I, *Cla* I and *Hae* III. Eventually, with restriction enzymes *Mse* I, *Tha* I and *Tsp* 509, the 11 isolates could be separated into two groups based on differential banding patterns (Glare and Inwood 1998). Therefore, in concordance with several other studies involving fungal species (Guillemaud et al. 1996; Persson et al. 1996; Bardin et al. 1997), restriction analysis of the ITS and 5.8*s* rRNA gene region was not suitable to discriminate closely related isolates within a species. Sequencing may be required to determine base pair differences in the ITS and 5.8*s* rRNA gene region if the ITS and 5.8*s* rRNA gene region is used to differentiate among isolates within a species.

This study identified only three clones that hybridized to repetitive sequences. Since nearly 200 recombinant clones were examined, these results suggest that *B. bassiana* contains little non-ribosomal repetitive DNA sequences. Fungal species have varied amounts of dispersed, non-ribosomal repetitive DNA sequences. For example, repetitive sequences constitute less than 1% of the genomes of *A. fumigatus, A. nidulans* and *Neurospora crassa* (Girardin et al. 1993; Timberlake 1978; Krumlauf and Marzluf 1980). In comparison, more than 25% of the genome of *M. graminicola* and *P. infestans* consisted of repetitive DNA (McDonald and Martinez 1990; Goodwin et al. 1992a). *Tolypocladium inflatum,* which is phylogenetically closely related to *Beauveria* spp. contains 1% non-rDNA repetitive sequences (Kempken et al. 1995). To our knowledge, no published study so far has examined the repetitive DNA content of entomopathogenic fungi.

In a previous study on *B. bassiana*, a low copy DNA sequence (pBb12) was identified from an isolate from *Melanoplus sanguinioes* (Hegedus and Khachatourians 1993a). By using pBb12, DNA polymorphisms were identified among isolates from different host species. Compared to probe pBb12, the non-ribosomal repetitive probe 133, which were developed in this study, generated more bands. Probe 133 distinguished *B. bassiana* isolates associated with fire ants, *Solenopsis* spp. from two commercial isolates that are associated with insect hosts of different orders. Another homologous repetitive DNA sequence (probe 205) generated results similar to those of probe 133. In conclusion, although only a limited number of repetitive DNA sequences were identified in *B. bassiana*, probes 133 and 205 were sufficient to distinguish the commercial isolates GHA and Naturalis from isolates collected from fire ants.

Rather than use dispersed and moderately repetitive DNA sequences, multilocus probes also can be developed by using known telomeric sequences as probes. Telomeric sequences have been found to be tandemly repeated DNA sequences at the end of chromosomes of all eukaryotes studied to date (Zakian 1989). Although telomeric sequences have been found to be diverse (McEachern and Blackburn 1994; Cohn et al. 1998), the short repeated sequence TTAGGG is shared by many fungi and animals (Blackburn 1984; Richards and Ausubel 1988; Powell and Kistler 1990). This study and previous studies indicate that *B. bassiana* isolates from different host species and geographic origins all contain the telomeric sequence TTAGGG (Viaud et al. 1996; Couteaudier and Viaud 1997).

DNA polymorphisms generated by the telomeric probe are due to repeated DNA sequences adjacent to the telomeric end of the chromosome, namely telomere-associated sequences (TAS). TAS are not as conserved as the telomeres themselves and often are polymorphic (McEachern and Blackburn 1994). Since every chromosome has two telomeres, multiple banding patterns can be observed when the genomic DNA is hybridized to telomeric repeated DNA sequences such as TTAGGG. In a previous study, RFLPs were observed among *B. bassiana* isolates from five host species (Viaud et al. 1996). In this study, $(\mathrm{TTAGGG})_{18}$ from *F. oxysporum* identified polymorphic DNA banding patterns among *B. bassiana* isolates from fire ants and two commercial product isolates. Both studies indicate that the probes containing repeated TTAGGG sequences are able to identify DNA polymorphisms in *B. bassiana*.

This study indicates that the mtDNA of *B. bassiana* is relatively conserved. Two fragments (20.0 kb and 1.8 kb) were observed among all tested isolates, when *F. oxysporum* mtDNA probe was hybridized to *Eco* RI- digested total DNA of *B. bassiana*. Also, major DNA bands were shared among all tested isolates, when the mtDNA probe was hybridized to *Hin*d III-digested genomic DNA. Similarly, when a 16.3 kb mtDNA-derived probe from the 28.5 kb mitochondrial genome of *B. bassiana* was hybridized to *Eco* RI- digested DNA from 15 isolates of *B. bassiana,* all isolates shared several bands (Pfeifer and Khachatourians 1989; Pfeifer et al. 1992a). Furthermore, using a 4.6 kb fragment from the 16.3 kb mtDNA region as a probe, 15 isolates could be separated into two types: four isolates shared a 4.1 kb band and eleven isolates shared a 5.0 kb band.

All tested isolates collected from fire ants had the same patterns, whereas GHA and Naturalis showed unique banding patterns. This suggests that mtDNA polymorphisms might be related to the host origin of isolates. In conclusion, heterologous mtDNA probes can be a suitable tool to distinguish isolates from fire ants from GHA and Naturalis.

In microbial control programs, one of the critical steps is to define discrete genetic characters for the isolates of interest. Most importantly, the characters must be stable and have enough resolution to meet specific needs. For registration requirements and patent protection, the characters need to distinguish the studied isolates from other isolates of the same species available on the market. Also, the methods for defining the characters should be rather simple, rapid and inexpensive. This study identified several molecular markers to distinguish Bb 447 from GHA and Naturalis, i. e. heterologous mtDNA, a heterologous telomeric probe and homologous RFLP probes 133 and 205. In the future, less costly, rapid PCR-based methods based on specific primers may be developed for faster isolate characterization.

CHAPTER 3

GENETIC DIVERSITY AMONG *BEAUVERIA BASSIANA* ISOLATES ASSOCIATED WITH FIRE ANTS, *SOLENOPSIS* SPP., AND OTHER HOST SPECIES

Introduction

Beauveria bassiana is perhaps the most extensively studied entomopathogenic fungus. This is mainly due to the fact that isolates of the species are used as microbial control agents against many insect species and *B. bassiana* has a wide host range, including over 200 different insect species belonging to nine orders. Hosts mainly belong to Lepidoptera and Coleoptera (Feng et al. 1994). A certain degree of host specificity among *B. bassiana* strains has been suggested (Fargues and Remaudiere 1977).

Understanding the relationships between hosts and *B. bassiana* genotypes is useful for biopesticide development. First, it helps to determine the possibility of selecting effective strains with defined genotypes as microbial control agents. Also, it can help to evaluate the host range for defined genotypes. Genetic diversity analysis of *B. bassiana* derived from target pests is fundamental for determining the host-genotype relationships. Specifically, genetic diversity analysis includes two aspects: first, the genetic diversity among isolates from the target host and; second, the genetic diversity and genetic distance between isolates from the target host and isolates from other host species.

Previous genetic diversity studies of *B. bassiana* mostly have focused on *B. bassiana* isolates from one host species. *B. bassiana* isolates studied with a variety of analytical tools displayed both genetic homogeneity and heterogeneity. Genetic homogeneity among *B. bassiana* isolates from the same host species was found among isolates from *Sitona humeralis* based on α-esterase isoenzyme profiles (Poprawski et al. 1988); from *Hypotherenmus hampei* according to electrophoretic and physiological profiles (Bridge et al. 1990); from *Ostrinia nubilalis* with vegetative compatibility group (VCG) analysis, telomeric fingerprinting, RFLPs and RAPDs (Couteaudier and Viaud 1997; Maurer et al. 1997a); and from *Diatraea saccharalis* and *Sitona discoideus* with RFLPs and RAPDs (Maurer et al. 1997a). On the other hand, genetic heterogeneity among *B. bassiana* isolates from the same host species was found among isolates from *S. discoideus* based on α-esterase isoenzyme profiles (Poprawski et al. 1988) and VCG analysis, and telomeric fingerprinting (Couteaudier and Viaud 1997). Other genetically diverse populations, including isolates from *Adephocoris lineolatus*, were found with α-esterase isoenzymes (Riba et al. 1986). Isolates from *Alphitobius diaperinus* differed in isoenzyme and RAPD analyses (Castrillo and Brooks 1998).

In addition, some studies examined isolates of *B. bassiana* from several host species rather than from a single host species. For example, high genetic heterogeneity was found among *B. bassiana* isolates from diverse host species (Mugnai et al. 1989; St. Leger et al. 1992). Isolates from related hosts from the same geographical areas appeared to be monomorphic when isolates were examined by API ZYM and esterase patterns (Mugnai et al. 1989).

In this study, single locus probes and repetitive probes were utilized to analyze genetic diversity among isolates collected from fire ants and other host species. Phylogenetic analysis was performed to determine host-genotype relationship of *B. bassiana* isolates associated with fire ants and other host species.

Materials and Methods

B. bassiana Isolates

The *B. bassiana* isolates listed in Table 3-1 were analyzed with single locus probes in order to elucidate the genetic diversity among isolates associated with fire ants, *Solenopsis* spp. Isolates listed in Tables 3-1 and 3-2 were used in the repetitive DNA probe analyses. Isolates from insects belonging to five orders and one isolate from soil listed in Table 3-3 were selected to compare the genetic variation between isolates from *Solenopsis* spp. and isolates from other hosts. The fungal culture was described in Chapter 2.

DNA Extraction, Choice of Restriction Enzymes and Probes

From each isolate, DNA was extracted by a CTAB extraction protocol described previously in Chapter 2. Also, the development of anonymous DNA probes from a genomic library of *B. bassiana* isolate 447 and the techniques related to RFLP analysis were described in Chapter 2. Seven single-locus probes, repetitive DNA probe 133 and the telomeric probe from *Fusarium oxysporum* were hybridized to Southern blots of *Hin*d III digested DNA to find RFLPs among isolates associated with *Solenopsis* spp. (Table 3-4). Furthermore, four additional single locus probes were hybridized to blots of DNA digested individually with multiple restriction enzymes (*Eco* RI, *Hin*d

Table 3-1. *Beauveria bassiana* isolates from fire ants and nest soil for testing single locus and repetitive probes

Isolates	**Origin**	**Host or Substrate**
Bb 447	Cuiaba, MT, Brazil	*Solenopsis invicta*
Bb 479	Cuiaba, MT, Brazil	*Solenopsis invicta*
Bb 591	Cuiaba, MT, Brazil	*Solenopsis invicta*
Bb 604	Cuiaba, MT, Brazil	Nest soil
Bb 662	Cuiaba, MT, Brazil	*Solenopsis* sp.
Bb 614	Lavras, MG, Brazil	*Solenopsis saevissima*
Bb 616	Lavras, MG, Brazil	*Solenopsis saevissima*
Bb 643	Lavras, MG, Brazil	Nest soil
Bb 649	Alambari, Sao Paulo, Brazil	*Solenopsis* sp.
Bb 899	Rio Grande do Sul, Brazil	*Solenopsis quinquecuspis*
Bb 903	Rio Grande do Sul, Brazil	*Solenopsis saevissima*
Bb 878	Colonia, Uruguay	*Solenopsis quinquecuspis*
Bb 882	Salinas, Uruguay	*Solenopsis richteri*
Bb 883	Punta del Este, Uruguay	*Solenopsis quinquecuspis*
Bb 886	Punta del Este, Uruguay	*Solenopsis invicta*
Bb 891	Rocha, Uruguay	*Solenopsis quinquecuspis*
Bb $7A_{10}$	Bakersfield, California	*Solenopsis xyloni*
Bb $7G_9$	Bakersfield, California	*Solenopsis xyloni*
Bb $9H_{11}$	Bakersfield, California	*Solenopsis xyloni*
Bb 876	Colonia, Uruguay	*Solenopsis quinquecuspis*
Bb 888	Punta del Este, Uruguay	*Solenopsis invicta*
Bb 896	Parque Stanta Tresesa, Uruguay	*Solenopsis invicta*
Bb 901	Rio Grande do Sul, Brazil	*Solenopsis saevissima*
Bb $7E_9$	Bakersfield, California	*Solenopsis xyloni*
Bb $9B_{10}$	Bakersfield, California	*Solenopsis xyloni*
Bb 542	Cuiaba, MT, Brazil	*Solenopsis invicta*
Bb 608	Cuiaba, MT, Brazil	Nest soil
Bb 434	Cuiaba, MT, Brazil	*Solenopsis invicta*
FP12C1	Gainesville, Florida	*Solenopsis invicta*
Bb 485	Piracicaba, Sao Paulo, Brazil	*Solenopsis saevissima*
Bb 555	Cuiaba, MT, Brazil	*Solenopsis invicta*
Bb 900	Rio Grande do Sul, Brazil	*Solenopsis quinquecuspis*
7421	Gainesville, FL	Nest soil
Bb 440*	Cuiaba, MT, Brazil	*Solenopsis invicta*
Bb 541*	Cuiaba, MT, Brazil	*Solenopsis invicta*

* Isolates used in experiments described in Table 3-6.

Table 3-2. Genetic variation of *Beauveria bassiana* isolates from *Solenopsis* spp. based on repetitive probe 133 and the telomeric probe in conjunction with *Pst* I

Isolates	**Origin**	**Host**	**133**	**Telo**
Bb 590	Piracicaba, Sao Paulo, Brazil	*Solenopsis saevissima*	1[1]	1
Bb 616	Lavras, MG, Brazil	*Solenopsis saevissima*	1	1
Bb 651	Alambari, Sao Paulo, Brazil	*Solenopsis* sp.	1	1
Bb 878	Colonia, Uruguay	*Solenopsis quinquecuspis*	1	1
Bb 879	Colonia, Uruguay	*Solenopsis quinquecuspis*	1	1
Bb 881	Salinas, Uruguay	*Solenopsis richteri*	1	1
Bb 883	Punta del Este, Uruguay	*Solenopsis quinquecuspi*	1	1
Bb 887	Punta del Este, Uruguay	*Solenopsis invicta*	1	1
Bb 888	Punta del Este, Uruguay	*Solenopsis invicta*	1	1
Bb 893	Parque Sta Tresesa, Uruguay	*Solenopsis invicta*	1	1
Bb 896	Parque Sta Tresesa, Uruguay	*Solenopsis invicta*	1	1
Bb 899	Rio Grande do Sul, Brazil	*Solenopsis quinquecuspi*	1	1
FP12C1	Gainesville, Florida	*Solenopsis invicta*	Non	2
SP111	Gainesville, Florida	*Solenopsis invicta*	1	1

[1] Numbers indicate DNA banding patterns.
Every column has an independent numbering system. The same number within the same column represents the same pattern. "Non" means DNA bands were not visible.

Table 3-3. DNA banding patterns generated by repetitive probes with *Hin*d III-digested DNA of *Beauveria bassiana* isolates collected from different hosts

Isolate	**Origin**	**Host**	**Probe 133**	**Telo. probe**
5715	Florida	*Scapteriscus vicinus* (Orthoptera: Gryllotalpidae)	1*	1
5781	Gainesville, Florida	Hemiptera	2	2
5782	Gainesville, Florida	Coleoptera	3	3
5783	Alachua, Florida	Soil	4	4
5792	Homestead, Florida	*Cylas formicarius elegantulus* (Coleoptera: Apionidae)	5	5
5818	La Molina, Peru	*Cylas formicarius elegantulus* (Coleoptera: Apionidae)	6	6
5820	Carabayllo, Peru	*Cylas formicarius elegantulus* (Coleoptera: Apionidae)	6	7
5822	Huancayo, Peru	*Premnotryes latithorax* (Coleoptera: Curculionidae)	7	8
5823	Huamachuco, Peru	*Premnotryes latithorax* (Coleoptera: Curculionidae)	Non	9
5838	Gainesville, Florida	Symphyta spp.	4	4
5849	Gainesville, Florida	*Metamasius callizona* (Coleoptera: Curculionidae)	8	10
5873	Lake Apopka, Florida	*Simyra henrici* (Lepidotera:Noctuidae)	9	11
5888	Gainesville, Florida	*Apis mellifera* (Hymenoptera: Apidae)	10	12
MVT	Spain	*Trachemys scripta*	11	13
BEar	Gainesville, Florida	Dermaptera	Non	14
Naturalis	Rio Grande Valley, Texas	*Anthonomus grandis grandis* (Coleoptera: Curculionidae)	12	15
GHA	Montana	*Melanoplus sanguinipes* (Orthoptera: Acrididae)	13	16

*Every column has an independent numbering system. The same number within the same column represents the same pattern. “Non” means DNA bands were not visible.

Table 3-4. Comparison of DNA pattern banding types generated by single-locus and repetitive probes with *Hin*d III- digested DNA of *Beauveria bassiana* isolates from fire ants and nest soil

Isolate	116^1	202^1	305^1	418^1	419^1	510^1	805^1	133	Telo.
Bb 447^2	1^3	1	1	-	1	1	-	1	1
Bb 479	1	1	1	-	1	1	-	1	1
Bb 591	1	1	1	1	-	-	1	1	1
Bb 604^2	1	1	1	1	-	-	1	1	2
Bb 662	1	1	1	-	1	1	-	1	1
Bb 614	1	1	1	1	-	-	1	1	2
Bb 616	1	1	1	1	-	-	1	1	1
Bb 643	1	1	1	1	-	-	1	1	1
Bb 649	1	1	1	1	-	-	1	1	2
Bb 899	1	1	1	1	-	-	1	1	1
Bb 903	1	1	1	1	-	-	1	1	1
Bb 878	1	1	1	1	-	-	1	1	1
Bb 882	1	1	1	1	-	-	1	1	1
Bb 883	1	1	1	1	-	-	1	1	1
Bb 886	1	1	1	-	1	1	-	1	1
Bb 891	1	1	1	1	-	-	1	1	1
Bb 7A$_{10}$	1	1	1	-	1	1	-	1	1
Bb 7G$_9$	1	1	1	-	1	1	-	1	1
Bb 9H$_{11}$	1	1	1	-	1	1	-	1	1
Bb 876^2	1	1	1	-	2	1	-	2	3
Bb 888	1	1	1	-	2	1	-	2	3
Bb 896	1	1	1	1	-	-	1	2	3
Bb 901	1	1	1	-	2	1	-	2	3
Bb 7E$_9$	1	1	1	-	2	1	-	2	3
Bb 9B$_{10}$	1	1	1	-	2	1	-	2	3
Bb 608^2	1	1	1	1	-	-	1	3	4
Bb 542^2	2	2	2	-	1	2	-	4	5
Bb 434^2	F	F	F	2	-	-	1	Non	6
FP12C1^2	F	F	F	3	-	-	1	Non	7
Bb 485^2	-	-	-	-	-	-	-	1	8
Bb 555^2	-	-	-	-	-	-	-	Non	9
Bb 900^2	-	-	-	-	-	-	-	1	10
7421^2	-	-	-	-	-	-	-	5	11

[1] Single-locus probes derived from a genomic library of *B. bassiana* isolate 447.

[2] Representative isolates with different DNA banding patterns were selected for UPGMA analysis and parsimony analysis.

[3] Numbers indicate specific DNA banding patterns. Every column has an independent numbering system. The same number represents the same pattern. Dash means no data, "Non" means DNA bands were not visible, "F" means very faint bands were visible.

III and *Pst* I) in order to detect polymorphisms (Table 3-5). Also, probe 133 and the telomeric probe were hybridized to DNA blots, which were individually digested with restriction enzymes *Pst* I, *Bam* HI, *Eco* RV and *Hae* III to detect additional polymorphisms among isolates associated with *Solenopsis* spp. (Tables 3-2 and 3-6). Polymorphisms among isolates collected from other insect species were detected with probe 133 and the telomeric probe by hybridizing to *Hin*d III digested DNA.

Data Analysis

Each single-locus probe-enzyme combination was assumed to define an RFLP locus. DNA fragments with different sizes were treated as alleles at each RFLP locus. In the DNA fingerprint study, only bands that could be scored unambiguously were included in the analysis. The presence or absence of bands was scored as binary data (1 for presence and 0 for absence of each band). Only representative isolates with different DNA banding patterns were selected for phylogenetic analysis (see Tables 3-3 and 3-4). In the phenetic approach, cluster analysis by the unweighted pair group arithmetic mean method (UPGMA) employed PAUP 4.0b (Illinois Natural History survey, Champaign, IL). Binary data generated by the telomeric sequence from *F. oxysporum* and repetitive probe 133 were used to determine the genetic similarity between isolates. In the cladistic approach, optimal trees were defined with the heuristic search option, and statistical confidence was assigned to hypotheses of relationships with bootstrap analysis.

Table 3-5. Genetic homogeneity among *Beauveria bassiana* isolates from *Solenopsis* species digested with *Eco* RI, *Hin*d III and *Pst* I

	***Eco* RI**				***Hin*d III**			***Pst* I**			
Isolate	**501**[1]	**520**[1]	**805**[1]	**Mt**[2]	**501**	**520**	**Mt**	**501**	**520**	**805**	**Mt**
Bb 447	1[3]	1	1	1	1	1	1	1	1	1	1
Bb 614	1	1	1	1	1	1	1	1	1	1	1
Bb 883	1	1	1	1	1	1	1	1	1	1	1
Bb 7G$_9$	1	1	1	1	1	1	1	1	1	1	1

[1] Probes derived from a genomic library of *B. bassiana* isolate 447.

[2] Mitochondrial DNA of *Fusarium oxysporum* used as heterologous probe.

[3] Numbers indicate DNA banding patterns generated by the specific probe-enzyme combination. Every column has an independent numbering system. The same number within the same column represents the same pattern.

Table 3-6. Genetic variation of *Beauveria bassiana* isolates from *Solenopsis* spp. digested with *Bam* H I, *Eco* RV and *Hae* III and hybridized to repetitive probe 133 and the telomeric probe

	***Bam* H I**		***Eco* R V**		***Hae* III**	
Isolate	**133**	**Telo.**	**133**	**Telo.**	**133**	**Telo.**
Bb 434	Non[1]	2	Non	2	Non	3
Bb 440	2	3	3	3	-	-
Bb 447	1	1	1	1	1	1
Bb 541	1	1	1	1	1	1
Bb 614	1	4	1	1	2	2
Bb 662	-	-	-	-	1	1
Bb 878	1	1	1	1	1	1
Bb 883	1	5	1	1	1	1
Bb 888	1	1	1	1	1	1
Bb 896	-	-	-	-	1	1
Bb 899	-	-	-	-	1	1

[1] Numbers indicate specific DNA banding patterns generated by probe-enzyme combinations. Every column has an independent numbering system. The same number within the same column represents the same pattern. "Non" means DNA band was not visible and dash means no data.

Results

Genetic Diversity among *B. bassiana* Isolates Associated with *Solenopsis* spp.

When seven single-locus probes were hybridized to *Hin*d III-digested DNA, only probe 419 was polymorphic (two alleles) among 26 isolates collected from five *Solenopsis* species in 10 locations (Table 3-4). In addition to these 26 isolates, three isolates, Bb 542, Bb 434 and FP12C1 showed unique patterns with some single-locus probes. Four additional single-locus probes did not differentiate among four isolates when DNA of the isolates was digested with *Eco* RI, *Hin*d III or *Pst* I (Table 3-5).

When isolates associated with *Solenopsis* species were characterized with *Hin*d III/ probe 133 and *Hin*d III/the telomeric probe, isolates could be subgrouped by both probes (Table 3-4). The telomeric probe revealed three banding patterns that were shared among several isolates. Isolates Bb 608, Bb 542, Bb 434, FP12C1, Bb 485, Bb 555, Bb 900 and 7421 showed unique banding patterns with this probe. Probe 133 showed two banding patterns were common among the isolates examined. Isolate Bb 608, Bb 542, 7421 showed unique banding patterns. Probe 133 did not hybridize to Bb 434, FP12C1 and Bb 555 (Table 3-4). A low level of polymorphisms was found when DNA was digested with *Pst* I and hybridized to the two repetitive probes (Table 3-2). Only isolate FP12C1 was found to be different from the other isolates. When Probe 133 and the telomeric probe were hybridized to *Bam* HI, *Eco* RV or *Hae* III digested DNA, isolates Bb 434 and Bb 440 had different DNA banding patterns with both probes and all enzymes (Table 3-6 and Fig. 3-1). Isolates Bb 614 and Bb 883 were differentiated from other isolates when *Bam* HI digested DNA was hybridized to

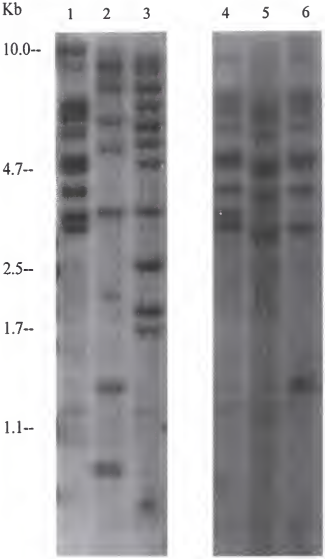


Fig 3-1. DNA fingerprinting profile of *Beauveria bassiana* isolates collected from *Solenopsis* spp. Lanes 1-6: Bb 878, Bb 434, Bb 440, Bb 541, Bb 614, and Bb 883. Genomic DNA was digested with *Bam* HI and hybridized to a telomeric probe from *Fusarium oxysporum.*

the telomeric probe. In addition, isolate Bb 614 was distinguished from other isolates when *Hae* III digested DNA was hybridized to probe 133 and the telomeric probe.

Comparison of Genetic Variation between Isolates from *Solenopsis* spp. and Isolates from Other Host Species

Only isolates 5783 and 5838 showed the same banding patterns with both the telomeric probe and probe 133 when hybridized to *Hin*d III-digested DNA (Table 3-3). In addition, isolate 5818 and 5820 displayed the same banding patterns with probe 133. DNA banding patterns of isolates from other hosts were not shared with those of isolates from *Solenopsis* spp. when probe 133 or the telomeric probe was hybridized to *Hin*d III-digested DNA (see Fig. 3-2 as an example). In dendrograms generated by UPGMA cluster analysis, isolates from *Solenopsis* spp. were grouped into several clusters based on banding patterns of *Hin*d III-digested DNA hybridized to the telomeric probe or to probe 133 (Figs. 3-3 and 3-4). For example, Bb 447, Bb 900, Bb 604 and Bb 876 formed a major cluster, and Bb 485 and Bb 555 were in a minor cluster (Fig. 3-3). Other isolates such as FP12C1, Bb 542, Bb 434, 7421 and Bb 608 were distant from each other. In parsimony analysis, poorly resolved trees were found by both data from the telomeric probe and probe 133 (Figs. 3-5 and 3-6). However, isolates from *Solenopsis* spp. that formed a major cluster in UPGMA analysis were grouped with strong bootstrap support (91%) when the data from the telomeric probe was used (Fig. 3-5).

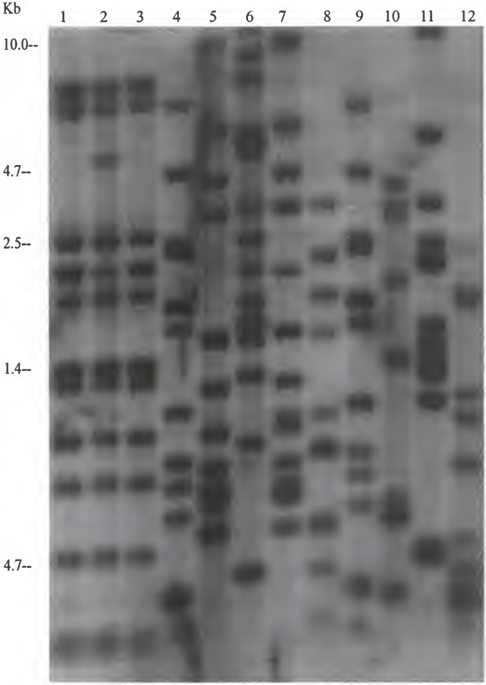


Fig 3-2. DNA fingerprinting profile of *Beauveria bassiana* isolates collected from *Solenopsis* spp. (lanes 1-3) and other insects (lanes 4-12). Lanes 1-12: Bb 899, Bb 900, Bb 903, Bb 5783, Bb 5818, Bb 5820, Bb 5822, Bb 5823, Bb 5838, Bb 5873, MVT and Bb 5792. Genomic DNA was digested with *Hind* III and hybridized to a telomeric probe from *Fusarium oxysporum*.

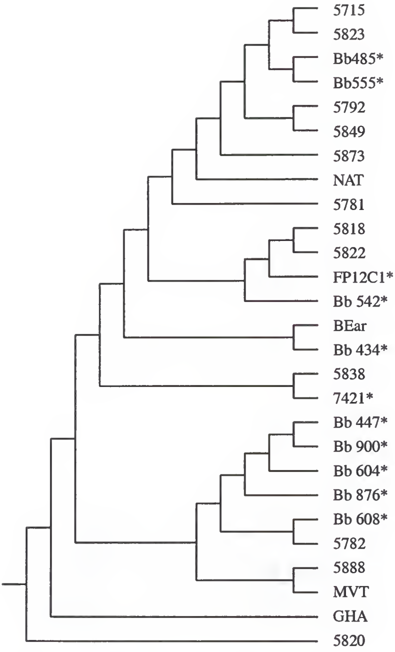


Fig. 3-3. Dendrogram showing the genetic relationships among *Beauveria bassiana* isolates. Cluster analysis was performed by the unweighted paired-group method with arithmetic mean (UPGMA). Similarity coefficients were based on 59 bands generated by the telomeric probe hybridized to *Hin*d III digested DNA. Asterisks indicate isolates that were collected from *Solenopsis* species or nest soil.

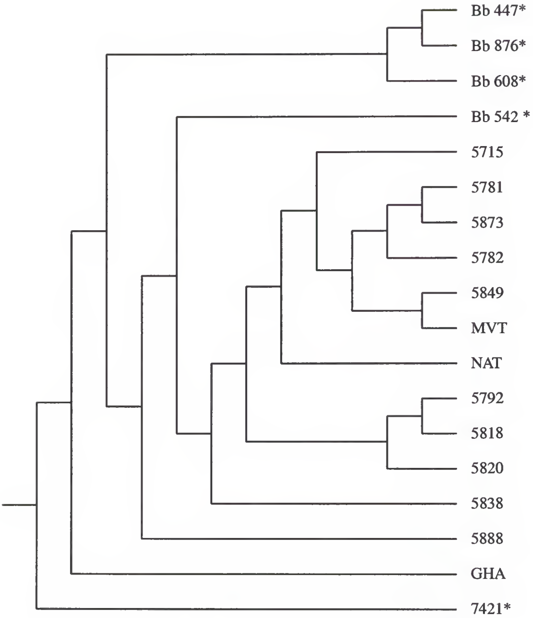


Fig. 3-4. Dendrogram showing the genetic relationships among *Beauveria bassiana* isolates. Cluster analysis was performed by the unweighted paired-group method with arithmetic mean (UPGMA). Similarity coefficients were based on 45 bands generated by probe 133 hybridized to *Hin*d III digested DNA. Asterisks indicate isolates that were collected from *Solenopsis* species or nest soil.

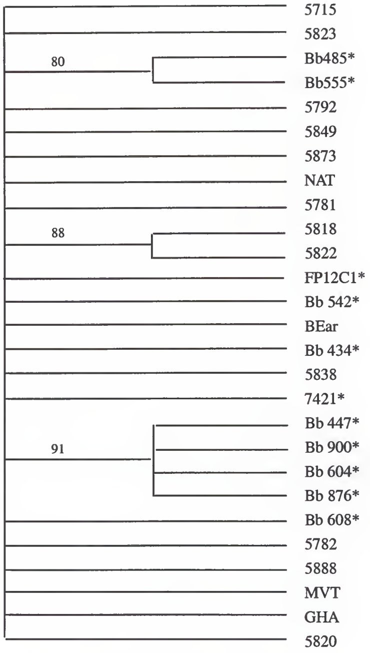


Fig. 3-5. Dendrogram showing the genetic relationships among *Beauveria bassiana* isolates. Cladistic approach was performed by parsimony analysis with the data from the telomeric probe. Number above branches represent bootsrap values based on 500 replicates. Only values greater than or equal to 50% are shown. Asterisks indicate isolates thatwere collected from *Solenopsis* species or nest soil.

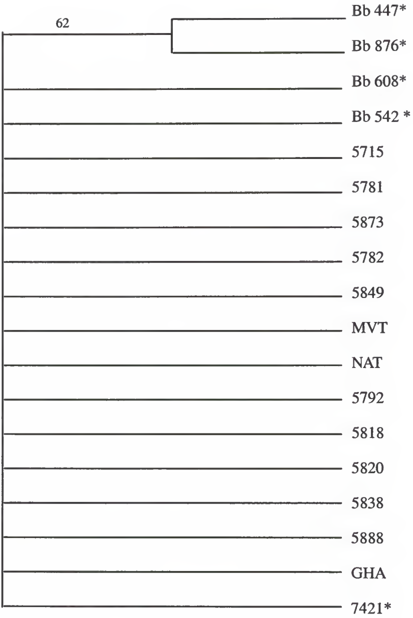


Fig. 3-6. Dendrogram showing the genetic relationships among *Beauveria bassiana* isolates. Cladistic approach was performed by parsimony analysis with the data from probe 133. Number above branches represent bootsrap values based on 500 replicates. Only values greater than or equal to 50% are shown. Asterisks indicate isolates that were collected from *Solenopsis* species or nest soil.

Discussion

Data generated by both probe 133 and the telomeric probe demonstrated that genetic diversity exists among *B. bassiana* isolates from *Solenopsis* species. Most isolates from *Solenopsis* species could be grouped with UPGMA analysis (Figs. 3-3 and 3-4) and parsimony analysis (Figs. 3-5 and 3-6). Genotypes belonging to the major cluster were found at a high frequency, indicating that isolates of this cluster may have a close association with *Solenopsis* species. Conversely, the low frequency of genotypes from other isolates suggests that these isolates might be newly adapted or not host-adapted. The major cluster displayed many genotypes with the telomeric probe (Figs. 3-3 and 3-5). This suggests that an ancestral host-adapted clone may have diversified by microevolutionary processes into a clonal lineage composed of genetically similar isolates. This is typical for predominate asexually reproducing fungi. Similarly, microevolution was strongly suggested in populations of *Candida albicans* to explain the limited differences of DNA banding patterns between genotypes which were identified by repetitive DNA probes (Lockhart et al. 1995, 1996).

Several lines of evidence produced by this study are consistent with the criteria of clonal reproduction established for eukaryotic microorganisms (Tibayrenc et al. 1990, 1991). First, identical genotypes were present and widespread. Genotypes were thoroughly defined by using up to four different restriction enzymes to digest DNA in conjunction with several single locus or repetitive DNA probes. Therefore, many loci were examined among isolates collected from *Solenopsis* species (Tables 3-2, 3-4 and 3-5). Second, based on the combined data from a single-locus probe (probe 419) and repetitive DNA probes (probe 133 and the telomeric probe), specific patterns were associated with specific lineages (Table 3-4). This indicates the presence of linkage disequilibrium, which is typical for asexual organisms.

That *B. bassiana* isolates from *Solenopsis* species have a predominant asexual reproduction is also supported by the fact that it was difficult to find polymorphic sites among isolates of the major group with single-copy DNA probes (Table 3-4). Generally, for fungal species that primarily use clonal reproduction, relatively little genetic diversity has been found among isolates originating from the same host species or from the same VCG. For example, while genetic diversity was high between isolates from different VCGs of *Fusarium oxysporum*, few polymorphisms were generally found within VCG (Tantaoui et al. 1996; Koenig et al. 1997). Similar results were described previously for *B. bassiana*. Based on six homologous RFLP probes and two known sequences, ten *B. bassiana* isolates from *O. nubilalis* were genetically homogeneous, while isolates from *S. discoideus* were genetically very similar (Maurer et al. 1997a).

While asexual reproduction is suggested as a predominant reproduction mode among *B. bassiana* isolates associated with *Solenopsis* spp., two examples in this study indicate that *B. bassiana* may not be a fungal species with exclusively clonal reproduction. First, the topologies of two trees from repetitive DNA probes were not concordant (Figs. 3-3 and 3-4; Figs. 3-5 and 3-6). Concordance of topologies is a feature for the species with a model of clonality. Also, poorly resolved trees in parsimony analysis with the data from two repetitive DNA probes indicate the possible existence of recombining population (Figs. 3-5 and 3-6).

In natural microbial populations, four models of population genetic structure have been proposed, based on reproductive strategy (Maynard Smith et al. 1993). So far, *B. bassiana* is considered as a deuteromycetous, haploid fungal species with no evidence of sexual reproduction. In addition, parasexual recombination was reported only after protoplast fusion in the laboratory (Kawanmoto and Aizawa 1986; Shimizu 1987; Paccola-Meirelles and Azevedo 1991) and has not been observed in natural populations. Recently, gene genealogy analysis with several species that were generally considered to reproduce exclusively by clonal reproduction showed that both reproduced clonally and sexually. Examples included *Aspergillus flavus* (Geiser et al. 1998), *Botryotinia fuckeliana* (Giraud et al. 1997), *Candida albicans* (Graser et al. 1996), and *Coccidioides immitis* (Burt et al. 1996; Koufopanou et al. 1997). Techniques with higher resolution such as sequencing specific regions of the genome may help to clarify the mode of reproduction for *B. bassiana* and to determine how sexual or parasexual processes influence genetic diversity.

Probe 133 did not hybridize to DNA from several isolates, including Bb 434, FC12C1, Bb 555, 5823, BEar. There are two possible explanations for this observation. First, this and other studies confirmed *B. bassiana* as being a highly heterogeneous species complex. Cluster analysis using RAPDs (Glare and Inwood 1998), isoenzyme (St. Leger et al. 1992) and biochemical analysis (Mugnai et al. 1989) demonstrated that other *Beauveria* species were dispersed among the *B. bassiana* species complex. Therefore, isolates which may have been morphologically identified as *B. bassiana* and which did not hybridize to probe 133 actually belong to "cryptic species" within the *B. bassiana* species complex.

A second explanation could be that repetitive DNA probe 133 may be a transposable element. In previous studies, some repetitive DNA sequences from fungi have been found to be transposable elements (Kistler and Miao 1992; Chen and Fonzi 1992; Dobinson et al. 1993; Daboussi and Langin 1994; Schmid-Berger et al. 1994; Glayzer et al. 1995; Langin et al. 1995). Recently, transposable elements were isolated from isolates of *B. bassiana* (Maurer et al. 1997b) and *B. nivea* (Kempken and Kuck 1996). If probe 133 is a transposable element, it can be present only among some but not all isolates of *B. bassiana.* For example, two repetitive sequences that were identified as transposable elements were present in 0 to 20 copies per genome among isolates of *Botrytis cinerea* (Diolez et al. 1995; Levis et al. 1997; Giraud et al. 1997). Since isolates that did not hybridize to probe 133 are associated with both *Solenopsis* spp. and other insect species, the second explanation seems to be the most likely.

Data generated in this study revealed that none of the isolates collected from other insect hosts were genetically similar to isolates from *Solenopsis* species, indicating that unique *B. bassiana* genotypes may be associated with *Solenopsis* species. Evidence from population studies of some fungal plant pathogens suggests the association of genetically defined groups within a fungal species with genetically defined groups of a host species. Fungal species include *Magnaporthe grisea* (Levy et al. 1991, 1993), *Fusarium oxysporum* (Kistler et al. 1991; Kistler 1995), *Sclerotinia sclerotiorum* (Anderson and Kohn 1995), *Phytophthora infestants* (Goodwin et al. 1995), and *Phaeoisariopsis griseola* (Guzman et al. 1996). In entomopathogenic insect fungi, *Entomophaga grylli* pathotypes, which have different host ranges for a grasshopper subfamily, could be genetically differentiated from each other by DNA probes (Bidochka et al. 1995, 1996). Also, RFLP analysis of the internal transcribed spacer regions separated *Beauveria brongniartii* isolates that have virulence against *Hoplochelus marginalis* from avirulent isolates (Neuveglise et al. 1994). In a previous *B. bassiana* study, which used RAPD and RFLP analyses, the insect host was suggested to be a predominant factor to explain some population structures (Maurer et al. 1997a). The nature of association of specific genotypes of *B. bassiana* associated with *Solenopsis* species will be more evident once more isolates are collected and analyzed.

Perfect association between a single host species and specific fungal genotypes can be possible only under monophyly for a single host species and exclusive clonal reproduction within the fungal species (Kistler 1995). Analysis in this study suggests that clonal reproduction may be a predominant but not exclusive reproduction mode within *B. bassiana.* Also, monophyletic or polyphyletic origin of *B. bassiana* genotypes associated with *Solenopsis* spp. can not be clarified based on the results from parsimony analysis in this study. Lastly, unlike other described fungi, the association of genotypes of *B. bassiana* with fire ants is determined at the genus level (*Solenopsis* spp.) and not at the species level. Therefore, even though this study demonstrated that certain lineages of *B. bassiana* associate with *Solenopsis* species, the association was not a perfect host-genotype relationship. Instead, some *B. bassiana* genotypes associated with *Solenopsis* species appear to be a *Solenopsis* genus-preferential clone lineage.

Previously, associations between *B. bassiana* isolates and specific insect hosts have been illustrated mainly by bioassays. Although host specificity of *B. bassiana* strains has been suggested (Fargues and Remaudiere 1977; Fargues et al. 1981), many studies showed that host-derived *B. bassiana* isolates are pathogenic to other insect species under relatively high inoculum levels used in laboratory bioassays (Ferron and Robert 1975; Fargues and Remaudiere 1977; Feng and Johnson 1990). For example, in another study of our project, no significant difference in mortality was observed when *S. invicta* was treated at a medium dosage with *B. bassiana* isolate 447 derived from *S. invicta* or isolate GHA, which originated from grasshopper. However, at either low or high dosage, higher mortality was caused by isolate 447. Thus, the efficiency of GHA to infect *S. invicta* in nature is probably low, when only a low dosage of isolate GHA is present. Integrating the observations of this study with the observations from bioassays with isolate 447 and GHA, it can be concluded that different genotypes of *B. bassiana* in nature may differ in their degree of host preference rather than be a strictly host specific.

Since *B. bassiana* isolates cannot be morphologically distinguished, bioassays have been the most common and direct method to evaluate the relationship between a certain insect host and a specific *B. bassiana* genotype. However, bioassays have two limitations. First, since *B. bassiana* is a facultative pathogen, it is impractical to test sufficient host species for evaluating the host-genotype relationships for isolates with unknown genotypes. Second, bioassays can only measure physiological specificity, and can hardly determine ecological specificity. In fact, ecological specificity is more critical when field performances of specific genotypes are evaluated. Therefore, genetically characterizing isolates and defining their genotypes might provide valuable insights about associations between *B. bassiana* genotypes and their ecological specificity to particular host species.

CHAPTER 4

GENETIC POPULATION STRUCTURE IN *BEAUVERIA BASSIANA* ASSOCIATED WITH FIRE ANTS, *SOLENOPSIS* SPP.

Introduction

The red imported fire ant (RIFA), *Solenopsis invicta,* was introduced into the United States from South America nearly 60 years ago and has become a very important pest in the southeastern regions of the United States. One of reasons for the rapid increase of *S. invicta* populations in the U.S. has been lack of the natural biological control that was found to control populations of *S. invicta* in the South American homeland (Lofgren 1986). After the failure of the RIFA eradication programs with chemical pesticides, researchers developed a variety of alternative control strategies (Talmadge 1980; Stimac and Alves 1994). One of the control strategies has been to introduce appropriate natural enemies, mainly from South America, as biological control agents. Many natural enemies have been found in the South American homeland of the RIFA (Jouvenaz et al. 1981). The entomopathogenic fungus *Beauveria bassiana* was discovered as a factor limiting *S. invicta* in Brazil (Stimac et al. 1987). Isolate 447 collected from *S. invicta* in Cuiaba, Mato Grosso, Brazil, was released in field experiments to control *S. invicta* in Florida and *S. xyloni* in California (Stimac and Alves 1994; Oi et al. 1994).

When a particular isolate of *B. bassiana* is selected as a biological control agent against a target host, understanding of genetic population structure is important to evaluate ecological adaptability of this isolate. Studies of genetic population structure in fungi so far have focused mainly on plant and human pathogens (Leung et al. 1993; McDonald and McDermott 1993; Milgroom 1995). Several studies of genetic population structure have been conducted in naturally occurring populations of *B. bassiana* with isoenzyme and RAPD analyses with relatively small sample sizes (Riba et al. 1986; Poprawski et al. 1988; Castrillo et al. 1999).

Both genetic homogeneity and heterogeneity were found among naturally occurring populations of *B. bassiana* from different host species. While genetic heterogeneity was observed among *B. bassiana* isolates from *Adelphocoris lineolatus* and *Sitona discoideus*, genetic homogeneity existed among isolates collected from *Lugus rugulipennis* and *S. humeralis* (Riba et al. 1986; Poprawski et al. 1988). High genetic heterogeneity was observed among isolates from *Alphitobius diaperinus* (Castrillo et al. 1999).

No study has been conducted to characterize genetic population structure of *B. bassiana* populations at the sites where the desired isolates were released. DNA techniques have been utilized to monitor released isolates of other entomopathogenic fungi in a new environment. RAPD analysis showed that released isolates of *Zoophthora radicans* could be recovered from infected leafhoppers after they were released (Hodge et al. 1995). Using pathotype-specific DNA probes, Bidochka et al. 1996 found that released isolates with a particular pathotype of *Entomophaga grylli* declined over years in infected grasshoppers. Since isolates of *B. bassiana* are morphologically indistinguishable, it is important to utilize DNA techniques to distinguish released isolates from possible indigenous isolates and to accurately evaluate the persistence of released isolates in new ecological conditions.

Repetitive fingerprinting probes have been used extensively to analyze fungal population structure. Species that have been studied in single fields or locations include *Mycosphaerella graminicola* (McDonald and Martinez 1991), *Cryphonectria parasitica* (Milgroom et al. 1992) and *Magnaporthe grisea* (Xia et al. 1993). Also, on a regional scale, population genetic studies have been conducted in *M. grisea* (Levy et al. 1991), *Phytophthora infestans* (Goodwin et al. 1992b and 1994; Drenth et al. 1993), and *Sclerotinia sclerotiorum* (Kohli et al. 1992 and Cubeta et al. 1997).

In this study, in order to understand the ecological adaptability of isolate 447, a telomeric probe and a repetitive DNA probe were utilized to describe genetic population structure of *B. bassiana* associated with *Solenopsis* spp. in a wide range of ecological conditions in South America. Also, *B. bassiana* isolates collected at two sites where isolate 447 was released and one site where no isolate was released in the United States were examined to further evaluate the ecological adaptability of isolate 447 in a new environment.

Materials and Methods

B. bassiana Isolates

Thirty-eight *B. bassiana* isolates, including isolate 447 from *S. invicta* in Cuiaba, Mato Grosso, Brazil, were collected in 1986 and 1987 and used for detailed population structure analysis (Table 4-1). In addition, isolates listed in Table 4-2 were a collection from *Solenopsis* spp. and nest soil in Uruguay and Brazil for further study of population structure. In the United States, two collections of *B. bassiana* isolates from *S. invicta* in Gainesville, Florida were analyzed (Table 4-3). At a sheep pasture

Table 4-1. Genetic variation among *Beauveria bassiana* isolates collected from *Solenopsis invicta* in Cuiaba, Mato Grosso, Brazil in 1986 and 1987

	Number of isolates	**Telomeric probe Type**	**Probe 133 Type**	**Frequency (%)**
1986				
	3	1t	1	50
	2	2t	2	33
	1	3t	0*	17
1987				
	27	1t	1	84
	1	2t	2	3
	1	3t	0	3
	3	4t	0	9

* "0" means the probe did not hybridize to the DNA of this isolate.

Table 4-2. Genetic variation among *Beauveria bassiana* isolates from *Solenopsis* species or nest soil in different locations

Isolates	Origin	Host	Probe 133 Type	Telomeric Type
Bb 886	Punta del Este, Uruguay	*S. invicta*	1	1t
Bb 887	Punta del Este, Uruguay	*S. invicta*	1	5t
Bb 888	Punta del Este, Uruguay	*S. invicta*	6	6t
Bb 893	Parque Santa Tresesa, Uruguay	*S. invicta*	1	5t
Bb 896	Parque Santa Tresesa, Uruguay	*S. invicta*	6	6t
Bb 876	Colonia, Uruguay	*S. quinquecuspis*	6	6t
Bb 878	Colonia, Uruguay	*S. quinquecuspis*	1	1t
Bb 879	Colonia, Uruguay	*S. quinquecuspis*	1	1t
Bb 883	Punta del Este, Uruguay	*S. quinquecuspis*	1	1t
Bb 884	Punta del Este, Uruguay	*S. quinquecuspis*	1	1t
Bb 885	Punta del Este, Uruguay	*S. quinquecuspis*	1	1t
Bb 890	Rocha, Uruguay	*S. quinquecuspis*	1	1t
Bb 891	Rocha, Uruguay	*S. quinquecuspis*	1	1t
Bb 899	Rio Grande do Sul, Brazil	*S. quinquecuspis*	1	1t
Bb 900	Rio Grande do Sul, Brazil	*S. quinquecuspis*	1	7t
Bb 485	Piracicaba, Sao Paulo, Brazil	*S. saevissima*	1	8t
Bb 590	Piracicaba, Sao Paulo, Brazil	*S. saevissima*	1	5t
Bb 614	Lavras, MG, Brazil	*S. saevissima*	1	5t
Bb 616	Lavras, MG, Brazil	*S. saevissima*	1	1t
Bb 901	Rio Grande do Sul, Brazil	*S. saevissima*	6	6t
Bb 903	Rio Grande do Sul, Brazil	*S. saevissima*	1	1t
Bb 881	Salinas, Uruguay	*S. richteri*	1	1t
Bb 882	Salinas, Uruguay	*S. richteri*	1	1t
Bb 649	Alambari, Sao Paulo, Brazil	*Solenopsis* sp.	1	5t
Bb 651	Alambari, Sao Paulo, Brazil	*Solenopsis* sp.	1	5t
Bb 662	Cuiaba, MT, Brazil	*Solenopsis* sp.	1	1t
Bb 760	Tramandai, RS	*Solenopsis* sp.	1	1t
Bb 604	Cuiaba, MT, Brazil	Nest soil	1	5t
Bb 608	Cuiaba, MT, Brazil	Nest soil	9	9t
Bb 643	Lavras, MG, Brazil	Nest soil	1	1t

Table 4-3. *Beauveria bassiana* isolates collected in Gainesville, Florida

Isolates	**Origin**	**Host**	**Probe 133 Type**	**Telomeric Probe Type**
SP 111	Sheep pasture, Gainesville, FL	*S. invicta*	1	1t
SPA112C1	Sheep pasture, Gainesville, FL	*S. invicta*	1	1t
SP32	Sheep pasture, Gainesville, FL	*S. invicta*	1	1t
SP3221	Sheep pasture, Gainesville, FL	Nest soil	1	1t
SP4721	Sheep pasture, Gainesville, FL	Nest soil	1	1t
SP8621	Sheep pasture, Gainesville, FL	Nest soil	1	1t
SP7421	Sheep pasture, Gainesville, FL	Nest soil	10	10t
FP12C1	Florida power, Gainesville, FL	*S. invicta*	0*	11t

* "0" means the probe did not hybridize to the DNA of this isolate.

site, isolate 447 was released the year before the collection. At the Florida Power collection site, no *B. bassiana* isolates have ever been released. In Bakersfield, California, one collection from the southern fire ant, *S. xyloni,* was examined (Table 4-4). Isolate 447 was released in Bakersfield, California in 1994. Prior to and after isolate 447 was released in 1995, isolates were collected for this study. *B. bassiana* isolates were cultured as described in Chapter 2.

DNA Extraction and Choice of Probes

DNA was extracted from each isolate by a CTAB extraction protocol described previously in Chapter 2. Since the telomeric sequence from *F. oxysporum* and the homologous repetitive probe 133 showed multiple banding patterns when each of them were hybridized to digested DNA of *B. bassiana* isolates (Chapter 2 and 3), these probes were used as DNA fingerprinting probes for isolate characterization. Methods associated with RFLP analysis were described previously in Chapter 2.

Results

Genetic Variation among *B. bassiana* Isolates Associated with *Solenopsis* spp.

Among six isolates collected from *S. invicta* in Mato Grosso, Brazil in 1986, three different genotypes were identified with the *Hin*d III-telomeric probe combination (Table 4-1). Three isolates, which included isolate 447 had 12 DNA bands ranging from 0.2 kb to 6.7 kb (Fig. 4-1A). This genotype was designated as genotype 1t. Two isolates were designated as genotype 2t (9 bands from 0.5 kb to 4 kb) and one isolate was designated as genotype 3t (13 bands from 0.3 kb to 7.2 kb) (see Fig. 4-1 B as an example). Four genotypes were identified among the collection from 1987. All three

Table 4-4. *Beauveria bassiana* isolates collected from *Solenopsis xyloni* in Bakersfield, California

	Isolates	Probe 133 Type	Telomeric probe Type
After the 1994 release and prior to the 1995 release			
	$7A_{10}$	1	1t
	$7C_6$	1	1t
	$7E_9$	6	6t
	$7G_9$	1	1t
	$9B_{10}$	6	6t
	$9F_8$	1	1t
	$9F_9$	1	1t
	$9H_{11}$	1	1t
After the 1995 release			
	$18A_{10}$	1	1t
	$18A_{12}$	1	1t
	$18B_1$	1	1t
	$18B_7$	1	1t
	$18D_6$	1	1t
	$19A_4$	6	6t
	$19B_8$	1	1t
	$19D_2$	6	6t
	$19D_7$	1	1t
	$19F_4$	1	1t

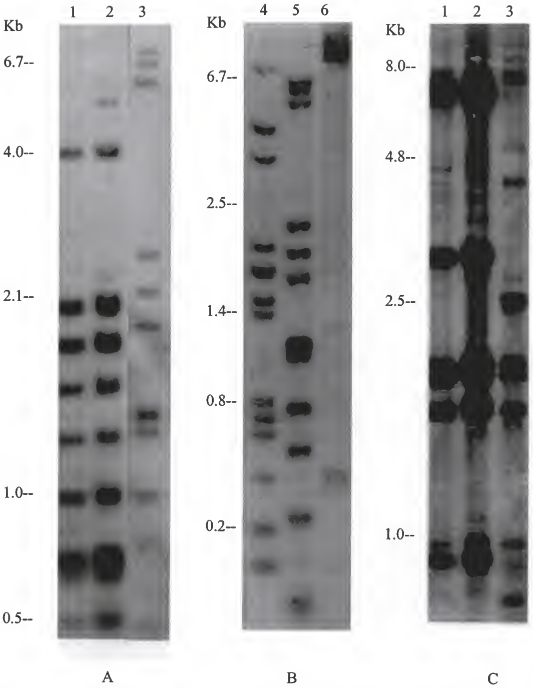


Fig. 4-1. Example of DNA banding patterns of *Beauvria bassiana* isolates collected from *Solenopsis invicta* in Cuiaba, Mato Grosso, Brazil in 1986 (A and C) and 1987 (B). Isolates in lanes 1-3 are Bb 440 (Genotype 2t or 2), Bb 445 (2t or 2) and Bb 446 (1t or 1). Isolates in lanes 4-6 are Bb 560 (3t), Bb 561 (1t) and Bb 562 (4t). Genomic DNA was digested with *Hin*d III and hybridized to the telomeric probe from *Fusarium oxysporum* (A and B) or probe 133(C).

telomeric genotypes (genotype 1t, 2t and 3t) previously found in the collection 1986 were recovered. Twenty-seven isolates among 32 tested (84%) showed genotype 1t. Genotype 2t and genotype 3t were found once in the 1987 collection (Table 4-1). An additional genotype displaying only two bands (8.7 kb and 9.4 kb) was identified and designated as genotype 4t (Fig. 4-1 B).

All isolates defined as genotype 1t in 1986 and 1987 collection were found to have the same banding pattern with probe 133 and therefore were designated as genotype 1 (Table 4-1). Genotype 1 defined by the combination probe 133/*Hin*d III had eight DNA bands ranging from 0.8 kb to 10.0 kb (Fig 4-1C). Similarly, isolates belonging to genotype 2t also displayed the same banding pattern with probe 133 and therefore were designated as genotype 2 (10 bands from 0.95 kb to 8 kb) (Table 4-1 and Fig. 4-1C). Isolates with genotype 3t and 4t did not hybridize to probe 133.

Genotype 1t or 1 was commonly found regardless of *Solenopsis* species and geographic location when *B. bassiana* isolates collected from four *Solenopsis* species or nest soil of fire ants in 10 locations were examined with the two repetitive probes (Table 4-2 and Fig. 4-2). Multiple genotypes were found among isolates from the same species in the same location. For example, two or three genotypes were found among the isolates collected from *S. invicta* in two locations of Uruguay (Table 4-2). In addition to genotype 1t or 1, genotypes 5t and 6t defined by the telomeric probe/*Hin*d III combination (Fig. 4-2) and genotype 6 defined by the 133/ *Hin*d III combination were identified (Fig. 4-3).

Among the isolates collected from *S. quinquecuspis*, two genotypes were identified from two locations (Colonia, Uruguay and Rio Grande do Sul, Brazil)

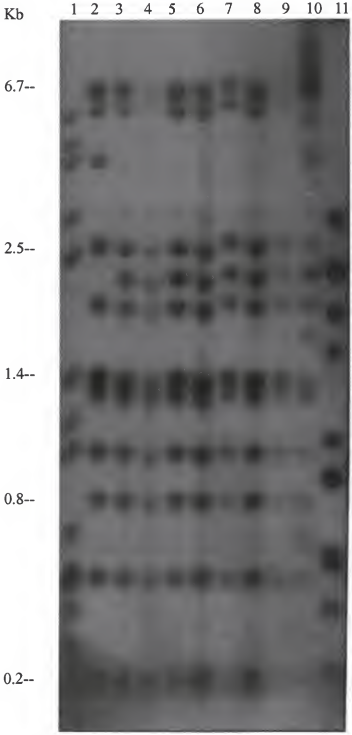


Fig. 4-2. Example of DNA banding patterns of *Beauvria bassiana* isolates collected from *Solenopsis* spp. in different locations (see Table 4-2). Isolates in lanes 1-11 are Bb 608 (Genotype 9t), Bb 893 (5t), Bb 899 (1t), Bb 903 (1t), Bb 878 (1t), Bb 882 (1t), Bb 883 (1t), Bb 886 (1t), Bb 891 (1t), Bb 896 (6t) and FP12C1 (11t). Genomic DNA was digested with *Hin*d III and hybridized to a telomeric probe from *Fusarium oxysporum*.

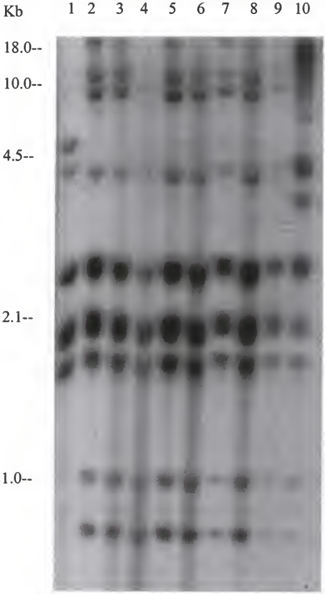
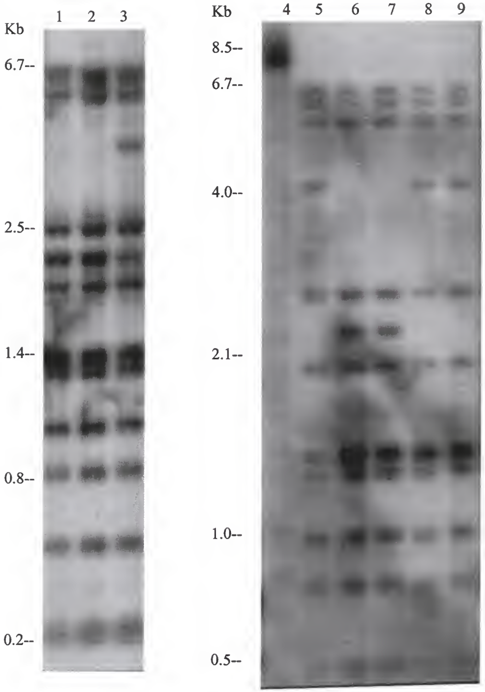


Fig. 4-3. Example of DNA banding patterns of *Beauvria bassiana* isolates collected from *Solenopsis* spp. in different locations (see Table 4-2). Isolates in lanes 1-10 are Bb 608 (Genotype 9), Bb 893 (1), Bb 899 (1), Bb 903 (1), Bb 878 (1), Bb 882 (1), Bb 883 (1), Bb 886 (1), Bb 891 (1), Bb 896 (6). Genomic DNA was digested with *Hin*d III and hybridized to probe 133.

(Table 4-2). Only one genotype (1, 1t) was identified in the other two locations (Punta del Este, and Rocha, Uruguay). Isolate Bb 900 from Rio Grande do Sul, Brazil, which was identified as genotype 1 by probe 133, was of genotype 7t defined by the telomeric probe/*Hin*d III combination. The banding pattern of genotype 7t was very similar to the one of genotype 1t, but had one additional 4.0 kb band (Fig. 4-4A). Among isolates collected from *S. saevissima*, different genotypes were identified by the telomeric probe/*Hin*d III combination for all three locations. For example, isolate Bb 485 displayed a unique genotype 8t with 2 bands (8 kb and 10 kb) (Fig. 4-4B). Probe 133 showed this isolate to be genotype 1. In addition, two isolates from *S. richteri* were of genotype 1 or 1t (Table 4-2). Also, the most common genotypes 1t and 5t were found among four isolates collected from unidentified *Solenopsis* spp. and two isolates collected from nest soil of fire ants (Table 4-2, Fig. 4-4B). Isolate Bb 608 from nest soil showed a unique genotype 9t with the telomeric probe (10 bands ranging from 0.45 kb to 5.6 kb) (Fig. 4-2), and genotype 9 using probe 133 (5 bands ranging from 1.8 kb to 5.6 kb) (Fig 4-3).

B. bassiana Isolates from *Solenopsis* spp. in the United States

Three isolates collected from *S. invicta* and three isolates collected from nest soil at the sheep pasture site in Gainesville, Florida were found to be genotype 1t and 1 (Table 4-3 and Fig. 4-5A). Meanwhile, isolate SP7421, which was isolated from nest soil was of genotype 10 (11 bands ranging from 0.8 kb to 9 kb). At the Florida Power site, only one isolate of *B. bassiana* (FP12C1) was found. This isolate had a unique genotype 11t (Fig. 4-2), with 10 bands ranging from 0.2 kb to 12 kb. Probe 133 did not hybridize to the DNA of this isolate.



A B

Fig. 4-4. Example of DNA banding patterns of *Beauvria bassiana* isolates collected from *Solenopsis quinquecuspis* (A), *S. saevissima* (B, lanes 4-5) and *Solenopsis* spp. (B, lanes 6-9) in different locations. Isolates in lanes 1-9 are: Bb 885 (1t), Bb 890 (1t), Bb 900 (7t), Bb 485 (8t), Bb 590 (5t), Bb 662 (1t), Bb 760 (1t), Bb 649 (5t) and Bb 651 (5t). Genomic DNA was digested with *Hin*d III and hybridized to a telomeric probe from *Fusarium oxysporum*.

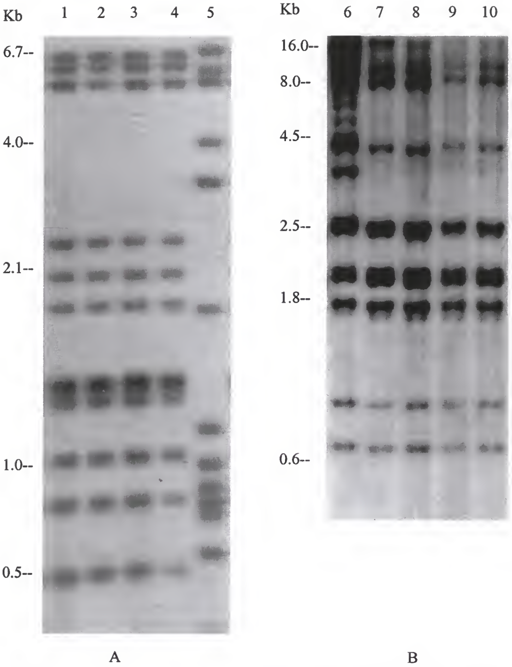


Fig. 4-5. Example of DNA banding patterns of *Beauvria bassiana* isolates collected from *Solenopsis invicta* or nest soil in Florida (A) and from *S. xyloni* in California (B). Isolates in lanes 1-10 are: SP 32 (1t), SP 12C1 (1t), SP 3221 (1t), SP 8621 (1t), SP 7421 (10t), 19D2 (6), 19F4 (1), 18B1 (1), 19D7 (1), 18D6 (1), 19B8 (1) and 18A12 (1). Genomic DNA was digested with *Hin*d III and hybridized to a telomeric probe from *Fusarium oxysporum* (A) and 133 probe (B).

At the Bakersfield, California site, isolates with genotype 1t and 6t defined by the telomeric probe/*Hin*d III combination or genotype 1 and 6 defined by the probe 133/ *Hin*d III combination were isolated from infected *S. xyloni* prior to and after the 1995 treatments (Fig. 4-5B). The frequency of genotype 1 was 75% and 80% prior to and after 1995 treatment, respectively (Table 4-4).

Discussion

This study demonstrated that multiple genotypes (defined by both homologous probe 133 and the heterologous telomeric probe) can be present among *B. bassiana* isolates collected from the same *Solenopsis* species in the same location. Genetic homogeneity and heterogeneity among *B. bassiana* isolates collected from the same host species in different studies are influenced by two factors: genetic markers and sample size.

Genetic markers may have different resolutions and therefore may influence the interpretation of population structure. DNA techniques normally have a higher resolution than allozymes (Bossart and Prowell 1998). Although both allozymes and DNA fingerprinting probes have been used to characterize the same *Phytophthora infestans* population, DNA fingerprinting was found to display a higher degree of resolution than allozyme analysis (Goodwin et al. 1992b). Also, two moderately repetitive DNA probes provided better resolution than multilocus enzyme electrophoresis, when highly related and unrelated *Candida albicans* isolates were examined (Pujol et al. 1997). In a recent study on *B. bassiana,* a higher level of genetic heterogeneity was observed among isolates from *Alphitobius diaperinus* by RAPDs than by isoenzyme analysis (Castrillo and Brooks 1998).

This study identified genotypes that were similar to each other which indicates that the repetitive DNA probe 133 and the telomeric probe have substantial resolution power to be used as DNA fingerprinting probes for differentiating isolates among and within clonal lineages. Both probes should be considered as good tools for describing genetic population structure. Two repetitive probes used in this study achieved a different level of resolution. Although probe 133 and the telomeric probe generated similar genotypes for *B. bassiana* isolates collected from *Solenopsis* spp., only the telomeric probe could hybridize to DNA from all tested *B. bassiana* isolates. Also, the telomeric probe was more sensitive in detecting genetic variation. For example, the isolates designated as genotype 1 by probe 133 were identified as four genotypes (1t, 5t, 7t and 8t) by the telomeric probe. Therefore, the telomeric probe is a tool with more discriminatory power to characterize isolates.

Sample size critically influences the interpretation of population structure. Unlike the relative ease of sampling of fungal plant pathogens from agricultural settings, the difficulty of sampling infected insect hosts is one of the main obstacles to population genetics studies of entomopathogenic fungi. In previous studies, a limited number of isolates were used to characterize populations of *B. bassiana* (Riba et al. 1986; Poprawski et al. 1988). In the present study, a sample with more than 30 isolates was collected in Cuiaba, Mato Grosso, Brazil. Although comprehensive studies, which examined genetic change of population structure over time, have been conducted for fungal plant pathogens (Chen et al. 1994; Kohli et al. 1995), the study of samples collected in Cuiaba was the first to examine genetic population structure in two consecutive years in entomopathogenic fungi. In both 1986 and 1987 collections, multiple genotypes were found and genotype 1/1t was predominant.

B. bassiana isolates with genotype 1/1t were predominant not only in detailed population studies of samples from the same location but also was found to be widespread on a large regional scale, as it was detected in many locations in South America. Genotype 1/1t was among *B. bassiana* isolates collected from different fire ant species or nest soil from the same location. For example, genotype 1/1t was predominant among isolates from *S. quinquecuspis* and *S. saevissima* in Rio Grande do Sul, Brazil and isolates from *S. invicta* and *S. quinquecuspis* in Punta del Este, Uruguay (Table 4-2). Also, genotype 1/1t was found among the same fire ant species collected in different locations: isolates from *S. invicta* in Mato Grosso, Brazil and Punta del Este, Uruguay; isolates from *S. quinquecuspis* in Rio Grande do Sul, Brazil as well as Colonia, Punta del Este, and Rocha, Uruguay; and isolates from *S. saevissima* in Minas Gerais and Rio Grande do Sul, Brazil. When the same genotypes in a population are recovered from more than 20 samples based on independent genetic markers, the population is considered to be a clonal population (Kohn 1994). By this criterion, the results of this study indicate that *B. bassiana* associated with *Solenopsis* spp. is a clonal population and has a predominate clone.

Similar observations were found in two plant pathogenic fungi when repetitive DNA sequences were used as fingerprinting probes. A single clonal lineage of *P. infestans* dominated most populations worldwide (Goodwin et al. 1994). In *S. sclerotiorum,* the most widely distributed clone was recovered over a distance of more than 2,000 km across Canada (Kohli et al. 1992). Also, globally distributed clonal lineages of *F. oxysporum* f. sp. *cubense* were found by using anonymous, single-copy RFLP probes (Koenig et al. 1997).

Unlike the wide distribution of clonal lineages of plant pathogenic fungi, which may be distributed for example *via* contaminated seed, it is not clear how specific *B. bassiana* genotypes associated with fire ants have spread so widely in South America. The spread of fire ants themselves might be one factor. Also, rivers located around fire ant habitats could transport infected ants. In addition, *B. bassiana* conidia may be dispersed by air.

The results generated by this study indicate that the frequency of genotype 1/1t may vary slightly, depending on geographic location and particular *Solenopsis* species. More specifically, genotype 1/1t was more frequent in Uruguay than in Brazil, regardless of *Solenopsis* host species. Also, in Punta del Este in Uruguay, isolates from *S. quinquecuspis* were observed at a higher frequency with genotype 1/1t than isolates from *S. invicta.*

In the United States, *Solenopsis* species were rarely infected with indigenous *B. bassiana* isolates. The only indigenous isolate of *B. bassiana* found in *S. invicta* is isolate FP12C1. Isolate FP12C1, which was collected at the Florida Power site in Gainesville, Florida, was identified as genotype 11. This genotype differed from all genotypes found from *Solenopsis* in South America. This suggests that genotype 11 was not introduced with *S. invicta* from South America to North America. Instead, genotype 11 probably originated from another insect host or substrate in Florida and therefore is probably not host-specific for fire ant. Furthermore, this study indicated indigenous isolates of *B. bassiana* in the U. S. do not infect fire ants as frequently as those assumed to have co-evolved with fire ants in South America. Another isolate, which was collected from nest soil and is considered as an indigenous isolate in the U. S., is isolate SP7421 of genotype 10. Genotype 10 also was different from any genotype found from *Solenopsis* spp. in South America.

At the sheep pasture in Florida where genotype 1/1t of isolate 447 was released, six out of seven isolates collected from *S. invicta* and from nest soil, were genotype 1/1t. This result demonstrates that the released isolates that originated from South America can persist at least one year in a new warm-temperate area in Florida.

In experiments at the Bakersfield, California site, *B. bassiana* 447 was released in 1994. Examined isolates were collected prior to and following release of isolate 447 in 1995. Among those isolates, both genotypes 1/1t and 6/6t were present. The isolates released in 1994 had been considered as only genotype 1/1t of isolate 447, but these isolates actually contained two genotypes (1/1t and 6/6t). Since genotype 6/6t has been detected only in South America, the recovery of genotype 6/6t suggests this genotype was unknowingly included in the release in 1994 in California. The results indicate that the released isolates originated from South America can successfully colonize in a arid area in California.

In conclusion, spatial and temporal observations in this study indicate that genotype 1/1t has a strong adaptability to survive in a wide range of ecological conditions and more importantly to successfully colonize in diverse environments in the U. S. Therefore, the development of isolate 447, which has genotype 1/1t, as a biological control agent for use against fire ants was justified by this study.

CHAPTER 5

SUMMARY AND CONCLUSIONS

Beauveria bassiana isolate 447, obtained in Brazil from *Solenopsis invicta,* has been evaluated in the United States as a microbial control agent against the fire ants *S. invicta* and *S. xyloni*. The main objective of this study was to genetically characterize isolate 447 and other *B. bassiana* isolates, including commercial product isolates. This study provided an example of how molecular techniques in conjunction with evolutionary and population genetics can be utilized for biopesticide development.

The limited DNA sequence heterogeneity of the ITS and 5.8*s* rRNA gene region was considered insufficient to efficiently discriminate among isolates using restriction analysis. Even though some heterogeneity in the ITS and 5.8*s* rRNA gene region was detected among *B. bassiana* isolate 447 and 14 other isolates from other insects, direct DNA sequencing of the ITS and 5.8*s* rRNA gene region was considered to be too expensive for routine isolate characterization, especially if a large number of isolates are to be examined. Due to the lack of polymorphisms among the restriction products between isolates, restriction analysis of the ITS and 5.8*s* rRNA gene region was not considered a feasible tool to differentiate isolates of *B. bassiana* for ecological studies.

Two homologous repetitive probes (probe 133 and probe 205) and two heterologous probes (a telomeric sequence probe and a mitochondrial probe both from *Fusarium oxysporum)* were able to distinguish isolate 447 from commercial product isolates GHA and Naturalis. Two combinations, the telomeric probe/*Hin*d III and probe 133/ *Hin*d III showed the highest level of discriminatory resolution among these three isolates. When the telomeric probe and probe 133 were used to examine more isolates confirmed as *B. bassiana* from *Solenopsis* spp. and other insect species, probe 133 hybridized to *Hin*d III-digested DNA from most but not all isolates tested. Conversely, the telomeric probe can be used to detect all tested isolates. Moreover, the telomeric probe demonstrated a better resolution than probe 133 for some isolates, especially the isolates from *Solenopsis* spp. Therefore, the telomeric probe/*Hin*d III has the most discriminatory power.

The telomeric probe/*Hin*d III alone or in combination with probe 133, probe 205 and the mitochondrial probe can be used as appropriate tools for developing isolate 447 as a biopesticide product. Unique molecular characters can be used to establish an identity for isolate 447 when EPA requires a product characterization for registration. The ability to distinguish isolate 447 from GHA and Naturalis by these tools also allows protection of the patent for a product if isolate 447 is used to formulate another product or if the products from other *B. bassiana* isolates are applied to control the target pests, which already have been patented for isolate 447 product.

The telomeric probe and probe 133 also were validated as an effective tool to ensure the accurate evaluation of field efficacy for released isolates. In the United States, *Solenopsis* species were rarely infected with indigenous *B. bassiana* isolates when three sites in Florida and California were surveyed. The only indigenous isolate, FP12C1 of genotype 11, was genetically very different from genotype 1/1t that is shared by isolate 447 and other genotypes from South America. Although additional sampling of *Solenopsis* species is necessary in the U.S. to confirm this initial observation, these results indicate that genotype 1/1t and other genotypes from South America may not be present in natural populations of *Solenopsis* in the United States. Therefore, when only genotypes 1/1t (or 1/1t and 6/6t), which commonly exist in South America, were found after releasing formulated isolates at a sheep pasture site in Florida and in almond orchards in Bakersfield, California, it indicated released isolates rather than possible indigenous genotypes should be responsible for the result of the treatment.

Discriminatory powers of the telomeric probe and probe 133 further allowed differentiation of very similar genotypes 1/1t and 6/6t at Bakersfield site in California. This indicates both probes can be used to examine the product and detect possible contamination during the production process. Product quality control will guarantee releasing the desirable genotypes to ensure the efficacy of the product and an appropriate monitoring of released genotypes.

Several results also indicate the possibility of using the telomeric probe and probe 133 as tools to monitor the environmental fate of released isolates associated with *Solenopsis* species. First, with both probes, none of the isolates from non-fire ant species was similar to isolates from *Solenopsis* species. This indicates that a unique *B. bassiana* group defined by the probes may be associated with *Solenopsis* species. Second, by using both probes, released isolates were recovered from the fields in Florida and California one year after release. Third, released genotypes in California were recovered from a site that is located 200 yards away from the previous release site.

When the genetic diversity of *B. bassiana* isolates from *Solenopsis* species and isolates from other insect species were compared, some *B. bassiana* genotypes associated with *Solenopsis* species appear to be a *Solenopsis* genus-preferential clone lineage. Therefore, molecular characterization of isolates of *B. bassiana* might be used to examine the association between specific isolate characteristics and their ecological specificity. When developing entomogenous pathogens as biological control agents, it is important to select appropriate genotypes that combine several superior characters, such as good production features, good field performance and an appropriate host range. Characterizing morphologically indistinguishable *B. bassiana* isolates with a combination of DNA probes and bioassay will help us better understand the potential of selected isolates in terms of physiological and ecological specificity as well as host range expansion.

Phylogenetic analysis suggests tha *B. bassiana* has a major lineage with isolates pathogenic to *Solenopsis* spp. The major clonal lineage consisted of many isolates displaying several similar genotypes and included genotype (1/1t) which was shared by isolate 447. The divergence of genetically similar genotypes in the major lineage suggests a co-evolutionary history between this lineage and *Solenopsis* species. Thus, genotypes belonging to this lineage, including isolate 447 are probably well adapted to *Solenopsis* species in diverse environments and therefore should be considered as the best candidates for the development of biological control agents against *Solenopsis* species.

The telomeric probe and probe 133 with several single locus probes as molecular markers were useful for clarifying the reproduction mode of *B. bassiana* associated with *Solenopsis* species in this study. Several lines of evidence produced by this study supported the view that asexual reproduction is a predominant mode of reproduction among *B. bassiana* isolates associated with *Solenopsis* spp. First, identical genotypes were present and widespread. Second, based on the combined data from a single-locus probe, probe 133 and the telomeric probe, specific patterns were associated with specific lineages. This indicates the presence of linkage disequilibrium. Third, little genetic variation was found among 29 *B. bassiana* isolates from five *Solenopsis* spp. On the other hand, two another results suggest that *B. bassiana* is not a fungal species with exclusively clonal reproduction. First, the topologies of two trees from repetitive DNA probes were not concordant. Also, the trees of parsimony analysis from the data of two repetitive DNA probes are poorly resolved.

Potentially effective isolates can be identified and evaluated by using DNA markers as a tool and an evolutionary and population genetics approach. The development of isolate 447 as a biological control agent for use against fire ants was justified by evidence generated in this study. When multiple genotypes were commonly found among *B. bassiana* isolates collected from the same species in the same locations, isolates with the same genotype as isolate 447 (1/1t) were predominant among isolates collected from different *Solenopsis* species and all of the surveyed geographic locations in South America. Also, while several *B. bassiana* genotypes collected from Mato Grosso, Brazil were present in both 1986 and 1987, genotype 1/1t was predominant in the population in both years. This strongly suggests that genotype 1/1t is well adapted to *Solenopsis* species at a genus level and is adaptable to proliferate a wide range of ecological conditions and possesses a high virulence against *Solenopsis* species. Another indication of the high general fitness of genotype 1/1t was that genotype 1/1t was recovered from two sites one year after release: the sheep pasture site in Gainesville, Florida, which is located in a warm temperate area and Bakersfield, California, which is an arid area. This suggests that genotype 1/1t may have strong adaptability to new ecological conditions.

In conclusion, this study established the methodology to characterize specific isolates of *B. bassiana.* This will help to fulfill identity requirements for registration and patent protection. Also, this may be useful when evaluating field efficacy evaluation of released isolates, controlling product quality and monitoring the fate of introduced fungal isolates in the environment. The study demonstrated how evolutionary and population genetics analyses can help to select potentially effective isolates with desirable ecological adaptability and other biological properties.

LIST OF REFERENCES

Alves, S. B., and Pereira, R. M. 1989. Production of *Metarhizium anisopliae* (Metsch) Sorok and *Beauveria bassiana* (Bals.) Vuill in plastic trays. Ecossistema 14: 188-192 (in Portuguese).

Anderson, J. B., and Kohn, L. M. 1995. Clonality in soilborne, plant-pathogenic fungi. Annu. Rev. Phytopathol. 33:369-91.

Anderson, T. E., Hajek, A. E., Roberts, D. W., Preisler, H. K., and Robertson, J. L. 1989. Colorado potato beetle (Coleoptera: Chrysomelidae): effects of combinations of *Beauveria bassiana* with insecticides. J. Econ. Entomol. 82: 83-89.

Anderson, T. E., and Roberts, D. W. 1983. Compatibility of *Beauveria bassiana* isolates with insecticide formulations used in Colorado potato beetle (Coleoptera: Chrysomelidae) control. J. Econ. Entomol. 76:1437-1441.

Anderson, T. E., Roberts, D. W., and Soper, R. S. 1988. Use of *Beauveria bassiana* for suppression of Colorado potato beetle populations in New York State (Coleoptera: Chrysomelidae). Environ. Entomol. 17:140-145.

Appel, D. J., and Gordon, T. R. 1994. Local and regional variation in populations of *Fusarium oxysporum* from agricultural field soils. Phytopathology 84:786-791.

Bardin, M., Nicot, P. C., Normand, P., and Lemaire, J. M. 1997. Virulence variation and DNA polymorphism in *Sphaerotheca fuliginea*, causal agent of powdery mildew of cucurbits. Eur. J. Plant Pathol. 103:545-554.

Bateman, R., Carey, M., Batt, D., Prior, C., Abraham, Y., Moore, D., Jenkins, N., and Fenlon, J. 1996. Screening for virulent isolates of entomopathogenic fungi against the desert locust, *Schistocerca gregaria* (Forskal). Biocontr. Sci. Technol 6:549-590.

Berretta, M. F., Lecuona, R. E., Zandomeni, R. O., and Grau, O. 1998. Genotyping isolates of the entomopathognic fungus *Beauveria bassiana* by RAPD with fluorescent labels. J. Invertebr. Pathol. 71:145-150.

Bidochka, M. J. McDonald, M. A., St. Leger, R. J., and Roberts, D. W. 1994. Differentiation of species and strains of entomopathogenic fungi by random amplification of polymorphic DNA (RAPD). Curr. Genet. 25:107-113.

Bidochka, M. J., Walsh, S. R. A., Ramos, M. E., St. Leger, R. J., Silver, J. C., and Roberts, D. W. 1995. Pathotypes in the *Entomophaga grylli* species complex of grasshopper pathogens differentiated with random amplification of polymorphic DNA and cloned DNA probes. Appl. Environ. Microbiol. 61: 556-560.

Bidochka, M. J., Walsh, S. R. A., Ramos, M. E., St. Leger, R. J., Silver, J. C., and Roberts, D. W. 1996. Fate of biological control introductions: monitoring an Australian fungal pathogen of grasshoppers in North America. Proc. Natl. Acad. Sci. USA. 93:918-921.

Bing, L. A., and Lewis, L. C. 1991. Suppression of *Ostrinia nubilalis* (Hubner) (Lepidoptera: Pyralidae) by endophytic *Beauveria bassiana* (Blasamo) Vuillemin. Environ. Entomol. 20:1207-1211.

Blackburn, E. H. 1984. Telomeres: do the ends justify the means? Cell 37: 7-8.

Bossart, J. L., and Prowell, D. P. 1998. Genetic estimates of population structue and gene flow: limitations, lessons and new directions. Tree 13:202-206.

Bradley, C. L., Black, W. E., Kearns, R., and Wood, P. 1992. Role of production technology in mycoinsecticide development. In *Frontiers in Industrial Mycology* (Leatham, G. F., Ed.). pp. 201-236. Chapman & Hall, New York.

Bridge, P. D., Abraham, Y. J., Cornish, M. C., Prior, C., and Moore, D. 1990. The chemotaxonomy of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) isolates from the coffee berry borer *Hypothenemus hampei* (Coleoptera: Scolytidae). Mycopathologia 111:85-90.

Bruns, T. D., White, T. J., and Taylor, J. W. 1991. Fungal molecular systematics. Annu. Rev. Ecol. Syst. 22:525-564.

Bulat, S. A., and Mironenko, N. V. 1996. Identification of fungi and analysis of their genetic diversity by polymerase chain reaction (PCR) with gene-specific and nonspecific primers. Russ. J. Genet. 32:143-159.

Burt, A., Carter, D. A., Koenig, G. L., White, T. J., and Taylor, J. W. 1996. Molecular markers reveal cryptic sex in the human pathogen *Coccidioides immitis*. Proc. Natl. Acad. Sci. USA. 93: 770-773.

Campbell, R. K., Anderson, T. E., Semel, M., and Roberts, D. W. 1985. Management of the Colorado potato beetle using the entomogenous fungus *Beauveria bassiana*. Am. Potato J. 62:29-37.

Castineiras, A., Pena, J. E., Duncan, R., and Osborne, L. 1996. Potential of *Beauveria bassiana* and *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) as biological control agents of *Thrips palmi* (Thysanoptera: Thripidae). Florida Entomol. 79:458-461.

Castrillo, L. A., and Brooks, W. M. 1998. Differentiation of *Beauveria bassiana* isolates from the darkling beetle, *Alphitobius diaperinus*, using isozyme and RAPD analyses. J. Invertebr. Pathol. 72:190-196.

Castrillo, L. A., Wiegmann, B. M. and Brooks, W. M. 1999. Genetic variation in *Beauveria bassiana* population associated with the darkling beetle, *Alphitobius diaperinus*. J. Invertebr. Pathol. (in press).

Chen, J. Y., and Fonzi, W. A. 1992. A temperature-regulated, retrotransposon-like element from *Candida albicans*. J. Bacteriol. 174:5624-5632.

Chen, R.S., Boeger, J. M. and McDonald, B. A. 1994. Genetic stability in a population of a plant pathogenic fungus over time. Mol. Ecol. 3:209-218.

Cohn, M., McEachern, M. J., and Blackburn, E. H. 1998. Telomeric sequence diversity within the genus *Saccharomyces*. Curr. Genet. 33:83-91.

Couteaudier, Y., Viaud, M., and Riba, G. 1996. Genetic nature, stability and improved virulence of hybrids from protoplast fusion in *Beauveria*. Microb. Ecol. 32:1-10.

Couteaudier, Y., and Viaud, M. 1997. New insights into population structure of *Beauveria bassiana* with regard to vegetative compatibility groups and telomeric restriction fragment length polymorphisms. FEMS Microb. Ecol. 22: 175-182.

Cubeta, M. A., Cody B. R., Kohli, Y., and Kohn, L. M. 1997. Clonality in *Sclerotinia sclerotiorum* on infected cabbage in eastern North Carolina. Phytopathology 87:1000-1004.

Daboussi, M. J., and Langin, T. 1994. Transposable elements in the fungal plant pathogen *Fusarium oxysporum*. Genetica 93:49-59.

De Hoog, G. S. 1972. The genera *Beauveria, Isaria, Tritirachium* and *Acrodontium* gen. nov. Stud. Mycol. (CBS) 1:1-41.

Diolez, A., Maeches, F., Fortini, D., and Brygoo, Y. 1995. Boty, a long-terminal-repeat retroement in the phytopathogenic fungus *Botrytis cinera*. Appl. Environ. Microbiol. 61:103-108.

Dobinson, K. F., Haris, R. E., and Hamer, J. E. 1993. *Grasshopper*, a long terminal repeat (LTR) retroelement in the phytopathgenic fungus *Magnaporthe grisea*. Mol. Plant-Microbe Interact. 6:114-126.

Drenth, A., Goodwin, S. B., Fry, W. E., and Dvvidse, L. C. 1993. Genotypic diversity of *Phytophthora infestants* in the Netherlands revealed by DNA polymorphisms. Phytopathology 83:1087-1092.

Dunn, P. H., and Mechalas, B. J. 1963. The potential of *Beauveria bassiana* (Blasamo) Vuillemin as a microbial insecticide. J. Invertebr. Pathol. 5:491-499.

Eyal, J., Mabud, M. D. A., Fischbei, K. L., Walter, J. F., Osborne, L. S., and Landa, Z. 1994. Assessment of *Beauveria bassiana* Nov. EO-1 strain, which produces a red pigment for microbial control. Appl. Biochem. Biotechnol. 44:65-80.

Fargues, J. D., Duriez-Vaucelle, T., Popeye, R. T., and Robert, P. H. 1981. Immunological characterization of the entmopathogenic hypomycetes *Beauveria* and *Metarrhizium*. Mycopathologia 75:101-108.

Fargues, J., and Remaudiere, G. 1977. Considerations of the specificity of entomopathogenic fungi. Mycopathologia 62:31-37.

Feng, M. G., and Johnson, J. B. 1990. Relative virulence of six isolates of *Beauveria bassiana* on *Diuraphis noxia* (Homoptera: Aphididae). Environ. Entomol. 19: 785-790.

Feng, M. G., Johnson, J. B., and Kish, L. P. 1990. Virulence of *Verticillium lecanii* and aphid-derived isolate of *Beauveria bassiana* (Fungi: Hyphomycetes) for six species of cereal-infesting aphids (Homoptera: Aphididae). Environ. Entomol. 19:815-820.

Feng, M. G., Poprawski, T. J., and Khachatourians, G. G. 1994. Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: current status. Biocontr. Sci. Technol. 4:3-34.

Ferron, P., and Robert, P. H. 1975. Virulence of entomopathogenic fungi (Fungi Imperfecti) for the adults of *Acanthoscelides obtectus* (Coleoptera: Bruchidae). J. Invertebr. Pathol. 25:379-387.

Ferron, P. 1981. Pest control by the fungi *Beauveria* and *Metarrhizium.* In *Mirobial Control of Pests and Plant Diseases 1970-1980* (Burges, H. D., Ed.), pp. 465-482. Academic Press, London.

Forster, H., Kinscherf, T. G., Leong, S. A., and Maxwell, D. P. 1989. Restriction fragment length polymorphisms of the mitochondrial DNA of *Phytophthora megasperma* isolated from soybean, alfalfa and fruit trees. Can. J. Bot. 67: 529-537.

Garber, R. C., and Yoder, O. C. 1984. Mitochondrial DNA of the filamentous ascomycete *Cochliobolus heterostrophus*. Curr. Genet. 8:621-628.

Gardes, M., Mueller, G. M., Fortin, J. A., and Kropp, B. R. 1991. Mitochondrial DNA polymorphisms in *Laccaria bicolor, L. laccata, L. proxima, and L. amethystina*. Mycol. Res. 95:206-216.

Geden, C. J., Rutz, D. A., and Steinkraus, D. C. 1995. Virulence of different isolates and formulation of *Beauveria bassiana* for house flies and the parasitoid *Muscidifurax raptor*. Biol. Contr. 5:615-621.

Geiser, D. M., Pitt, J. I., and Taylor, J. W. 1998. Cryptic speciation and recombination in the aflatoxin-producing fungus *Aspergillus flavus*. Proc. Natl. Acad. Sci. USA. 95:388-393.

Girardin, H., Latge, J. P., Srikantha, T., Morrow, B., and Soll, D. R. 1993. Development of DNA probes for fingerprinting *Aspergillus fumiganus*. J. Clin. Microbiol. 31:1547-1554.

Giraud, T., Fortini, D., Levis, C., Leroux, P., and Brygoo, Y. 1997. RFLP markers show genetic recombination in *Botryotinia fuckeliana* (*Botrytis cinerea*) and transposable elements reveal two sympatric species. Mol. Biol. Evol. 14:1177-1185.

Glare, T. R., and Inwood, A. J. 1998. Morphological and genetic charaterization of *Beauveria* spp. from New Zealand. Mycol. Res. 102:250-256.

Glayzer, D. C., Roberts, I. N., Archer, D. B., and Oliver, R. P. 1995. The isolation of *Ant l*, a transposable element from *Aspergillus niger*. Mol. Gen. Genet. 249: 432-438.

Goettel, M. S. 1992. Fungal agents for biocontrol. In *Biological Control of Locusts and Grasshoppers*. (Lomer, C. J. and Prior, C. D, Eds.), pp.122-132. CAB International, Wallingford, U. K.

Goettel, M. S., Johnson, D. L., and Inglis, G. D. 1995. The role of fungi in the control of grasshoppers. Can. J. Bot. 73 (Suppl. 1):71-75.

Goettel, M. S., Poprawski, T. J., Vandenberg, J. D., Li, Z., and Roberts, D. W. 1990. Safety to nontarget invertebrates of fungal biocontrol agents. In *Safety of Microbial Insecticides* (Laird, M., Lacey, L. A., and Davidson, E. W., Eds.), pp. 210-231. CRC Press, Boca Raton, FL.

Goodwin, S. B., Cohen, B. A., and Fry, W. E. 1994. Panglobal distributuion of a single clonal lineage of the Irish potato famine fungus. Proc. Natl. Acad. Sci. USA 91:11591-11595.

Goodwin, S. B., Drenth, A., and Fry, W. E. 1992a. Cloning and genetic analysis of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. Curr. Genet. 22:107-115.

Goodwin, S. B., Drenth, A., and Fry, W. E. 1992b. Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. Phytopathology 82:955-961.

Goodwin, S. B., Sujkowski, L. S., and Fry, W. E. 1995. Rapid evolution of pathogenicity within clonal lineages of the potato late blight disease fungus. Phytopathology 85:669-676.

Graser, Y., Volovsek, M., Arrington, J., Schonian, G., Presber, W., Mitchell, T. G., and Vilgalys, R. 1996. Molecular markers reveal that population structure of the human pathogen *Candida albicans* exhibits both clonality and recombination. Proc. Natl. Acad. Sci. USA. 93:12473-12477.

Guillemaud, T., Raymond, M., Callot, G., Cleyet-Marel, J. C., and Fernandez, D. 1996. Variability of nuclear and mitochondrial ribosomal DNA of a truffle species (Tuber aestivum). Mycol. Res. 100:547-550.

Guzman, P., Gilbertson, R. L., Nodari, R., Johnson, W. C., Temple, S. R., Mandala, D., Mkandawire, A. B. C., and Gepts, P. 1995. Characterization of variability in the fungus *Phaeoisariopsis griseola* suggests coevolution with the common bean (*Phaseolus vulgaris*). Phytopathology 85:600-607.

Hajek, A. E., Soper, R. S., Roberts, D. W., Anderson, T. E., Biever, K. D., Ferro, D. N., LeBrun, R. A., and Storch, R. H. 1987. Foliar applications of *Beauveria bassiana* (Blasamo) Vuillemin for control of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). An overview of pilot test results from the northern United States. Can. Entomol. 119:959-974.

Hajek, A. E., and St Leger, R. J. 1994. Interactions between fungal pathogens and insect hosts. Annu. Rev. Entomol. 39: 293-322.

Hall, R. A. 1982. Deuteromycetes: Virulence and bioassay design. Proc. 3rd Int. Colloq. Invertebr. Pathol. pp. 191-196. Brighton, U. K.

Hall, R. A. and Papierok, B. 1982. Fungi as biological control agents of arthropods of agricultural and medical importance. Parasitology 84:205-240.

Hamer, J. E., Farrel, L., Orbach, M. J., Valent, B., and Chumley, F. G. 1989. Host species-specific conservation of a family of repeated DNA sequences in the genome of a fungal plant pathogen. Proc. Natl. Acad. Sci. USA. 86:9981-9985.

Hayden, T. P., Bidochka, M. J., and Khachatourians, G. G. 1992. Virulence of several entomopathogenic fungi, and host-passage strains of *Pacilomyces farinosus*, toward the blackberry-cereal aphid *Sitobion fragariae*. J. Econo. Entomol. 85:58-64.

Hegedus, D. D., and Khachatourians, G. G. 1993a. Construction of cloned DNA probes for the specific detection of the entomopathogenic fungus *Beauveria bassiana* in grasshoppers. J. Invertebr. Pathol. 62:233-240.

Hegedus, D. D., and Khachatourians, G. G. 1993b. Identification of molecular variants in mitochondrial DNAs of members of the genera *Beauveria, Verticillium, Paecilomyces, Tolypocladium, and Metarhizium*. Appl. Environ. Microbiol. 59:4283-4288.

Hegedus, D. D., and Khachatourians, G. G. 1996. Identification and differentiation of the entomopathogenic fungus *Beauveria bassiana* using polymerase chain reaction and single-strand conformation polymorphism analysis. J. Invertebr. Pathol. 76:289-299.

Henrion, B., Chevalier, G., and Martin, F. 1994. Typing truffle species by PCR amplification of the ribosomal DNA spacers. Mycol. Res. 98:37-43.

Hibbett, D. S. 1992. Ribosomal RNA and fungal systematics. Trans. Mycol. Soc. Japan 33:533-556.

Hodge, K. T., Sawyer, A. J., and Humber, R. A. 1995. RAPD-PCR for identification of *Zoophthora radicans* isolates in biolgical control of the potato leafhopper. J. Invertebr. Pathol. 65:1-9.

Ignoffo, C. M., Garcia, C., Alyoshina, O. A., and Lappa, N. V. 1979. Laboratory and field studies with Boverin: a mycoinsecticideal preparation of *Beauveria bassiana* produced in the Soviet Union. J. Econ. Entomol. 72:562-565.

Inglis, G. D., Johnson, D. L., and Goettel, M. S. 1996. Effect of bait substrate and formulation on infection of grasshopper nymphs by *Beauveria bassiana*. Biocontr. Sci. Technol. 6:35-50.

Inglis, G. D., Johnson, D. L., and Goettel, M. S. 1997. Field and laboratory evaluation of two conidial batches of *Beauveria bassiana* (Balsamo) Vuillemin against grasshoppers. Can. Entomol. 129:171-186.

Jaronski, S. T. 1997. New paradigms in formulating mycoinsecticides. In *Pesticide Formulations and Application Systems:* 17th volume.(Goss, G. R., Hopkinson, M. J., and Collins, H. M. Eds.), pp. 99-112. ASTM, West Conshohocken, PA.

Jeng, R. S., Duchesne, L. C., Sabourin, M., and Hubbes, M. 1991. Mitochondrial DNA restriction fragment length polymorphisms of aggressive and non-aggressive isolates of *Ophiostoma ulmi.* Mycol. Res. 95:522-537.

Johnson, D. L., and Goettel, M. S. 1993. Reduction of grasshopper populations following field application of the fungus *Beauveria bassiana*. Biocontr. Sci. Technol. 3:165-175.

Johnson, J. A., Hall, I. M., and Arakawa, K. Y. 1984. Epizootiology of *Erynia phytonomi* (Zygomycetes: Entomophthorales) and *Beauveria bassiana* (Deuteromycetes: Moniliales) parasitizing the Egyptian alfalfa weevil (Coleoptera: Curculionidae) in southern California. Environ. Entomol. 13:95-99.

Jones, W. E., Grace, J. K., and Tamashiro, M. 1996. Virulence of seven isolates of *Beauveria bassiana* and *Metarhizium anisopliae* to *Coptotermes formosanus* (Isoptera: Rhinotermitidae). Environ. Entomol. 25:481-487.

Jouvenaz, D. P., Lofgren C. S., and Banks, W. A. 1981. Biological control of imported fire ants: a review of current knowledge. Bull. Entomol. Soc. Am. 27:203-208.

Kaaya, G. P., and Munyinyi, D. M. 1995. Biocontrol potential of the entomogenous fungi *Beauveria bassiana* and *Metarhizium anisopliae* for tsetse flies (*Glossina* spp.) at developmental sites. J. Invertebr. Pathol. 66:237-241.

Kaaya, G. P., Mwangi, E. N., and Ouna, E. A. 1996. Prospects for biological control of livestock ticks, *Rhipicephalus appendiculatus* and *Amblyomma variegatum*, using the entomogenous fungi *Beauveria bassiana* and *Metarhizium anisopliae*. J. Invertebr. Pathol. 67:15-20.

Kawanmoto, H., and Aizawa, K. 1986. Fusion conditions for protoplasts of an entomogenous fungus, *Beauveria bassiana*. Appl. Entom. Zool. 21:624-626.

Kempken, F., Schreiner, C., Schorgendorfer, and Kuck, U. 1995. A unique repeated DNA sequence in the cyclosprin-producing strain of *Tolypocladium inflatum* (ATCC 34921). Exp. Mycol. 19:305-313.

Kempken, F., and Kuck, U. 1996. Restless, an active *Ac*-like transposon from the fungus *Tolypocladium inflatum*: structure, expression, and alternative RNA splicing. Mol. Cell. Biol. 16:6563-6572.

Khachatourians, G. G. 1992. Virulence of five *Beauveria* strains, *Paecilomyces farinosus*, and *Verticillium lecanii* against the migratory grasshopper, *Melanoplus sanguinipes*. J. Invertebr. Pathol. 59:212-214.

Kistler, H. C. 1991. Genetic manipulation of plant pathgenic fungi. In: *Mirobial Control of Weeds* (TeBeest, D. O., ed.), pp.152-170, Routledge, Chapman and Hall, London.

Kistler, H. C. 1995. Genetic diversity in the plant-pathogenic fungus *Fusarium oxysporum*. Phytopathology 87:474-479

Kistler, H. C., and Benny, U. 1989. The mitochondrial genome of *Fusarium oxysporum*. Plasmid 22:86-89.

Kistler, H. C., Bosland, P. W., Benny, U., Leong, S., and Williams, P. H. 1987. Relatedness of strains of *Fusarium oxysporum* from crucifers measured by examination of mitochondrial and ribosomal DNA. Phytopathology 77: 1289-1293.

Kistler. H. C., and Miao, V. P. W. 1992. New modes of genetic change in filamentous fungi. Annu. Rev. Phytopathol. 30:131-52.

Kistler, H. C., Momol, E. A., and Benny, U. 1991. Repetitive genomic sequences for determining relatedness among strains of *Fusarium oxysporum*. Phytopathology 81:331-336.

Koenig, R. L., Ploetz, R. C., and Kistler, H. C. 1997. *Fusarium oxysporum*. f. sp. *cubense* consists of a small number of divergent and globally distributed clonal lineages. Phytopathology 87:915-923.

Kohli, Y., Brunner, L. J., Yoell, H., Milgroom, M. G., Anderson, J. B., Morral, R. A. A., and Kohn, L. M. 1995. Clonal dispersal and spatial mixing in populations of the plant pathogenic fungus, *Sclerotinia sclerotiorum*. Mol. Ecol. 4:69-77.

Kohli, Y., Morral, R. A. A., Anderson, J. B., and Kohn, L. M. 1992. Local and trans-Canadian clonal distribution of *Sclerotinia sclerotiorum* on canola. Phytopathology 82:875-880.

Kohn, L. M. 1992. Developing new characters for fungal systematics: an Experimental approach for determining the rank of resolution. Mycologia 84:139-153.

Kohn, L. M. 1995. The clonal dynamic in wild and agricultural plant-pathogen populations. Can. J. Bot. 73 (Suppl. 1):S1231-S1240.

Kohn, L. M., Stasovski, E., Carbone, I., Royer, J., and Anderson, J. B. 1991. Mycelial incompatibility and molecular markers identify genetic variability in field populations of *Sclerotinia sclerotiorum*. Phytopathology 81:480-485.

Kosir, J. M., Macpherson, J. M., and Khachatourians, G. G. 1991. Genomic analysis of a virulent and a less virulent strain of the entomopathogenic fungus *Beauveria bassiana,* using restriction fragment length polymorphisms. Can. J. Microbiol. 37:534-541.

Koufopanou, V., Burt, A., and Taylor, J. W. 1997. Concordance of gene genealogies reveals reproductive isolation in the pathogenic fungus *Coccidioides immitis*. Proc. Natl. Acad. Sci. USA 94:5478-5482.

Krumlauf, R., and Marzluf, G. A. 1980. Genome organization and characterization of the repetitive and inverted repeat DNA sequences in *Neurospora crassa*. J. Bio. Chem. 255:1138-1145.

Kurdyla, T. M., Guthrie, P. A. I., McDonald, B. A., and Appel, D. N. 1995. RFLPs in mitochondrial and nuclear DNA indicate low levels of genetic diversity in the oak wilt pathogen *Ceratocystis fagacearum*. Curr. Genet. 27:373-378.

Landa, Z., Osborne, L., Lopez, F., and Eyal, J. 1994. A bioassay for determining pathogenicity of entomogenous fungi on whiteflies. Biol. Contr. 4:341-350.

Langin, T., Capy, P., and Daboussi, M. J. 1995. The transposable element *impala,* a fungal member of the *Tcl-mariner* superfamily. Mol. Gen. Genet. 246:19-28.

Leathers, T. D., Gupta, S. C., and Alexander, N. J. 1993. Mycopesticides: status, challenges and potential. J. Industrial Microbiology 12:6975-6980.

Leung, H., Nelson, R. J., and Leach, J. E. 1993. Population structure of plant pathogenic fungi and bacteria. Adv. Plant Pathol. 10: 157-205.

Levis, C., Fortini, D., and Brygoo, Y. 1997. Flipper, a mobile Fot1-like transposable element in Botrytis cinerea. Mol. Gen. Genet. 254:674-680.

Levy, M., Romao, J., Marchetti, M. A., and Hamer, J. E. 1991. DNA fingerprinting with a dispersed repeated sequence resolves pathotype diversity in the rice blast fungus. Plant Cell 3:95-102.

Levy, M., Corea-Victoria, F. J., Zeigler, R. S., Xu, S., and Hamer, J. E. 1993. Genetic diversity of the rice blast fungus in a disease nursery in Colombia. Phytopathology 83:1427-1433.

Limber, D. B. 1940. A new form genus of the Moniliaceae. Mycologia 32:23-30.

Lockhart, S. R., Fritch, J. J., Meier, A. S., Schroppel, K., Srikantha, T., Galask, R., and Soll, D. R. 1995. Colonizing populations of *Candida albicans* are clonal in origin but undergo microevolution through C1 fragment reorganization as demonstrated by DNA fingerprinting and C1 sequencing. J. Clin. Microbiol. 33:1501-1509.

Lockhart, S. R., Reed, B. D., Pierson, C. L., and Soll, D. R. 1996. Most frequent scenario for recurrent *Candida* vaginitis is strain maintenance with "substrain shuffling": demonstration by sequential DNA fingerprinting with probes. J. Clin. Microbiol. 34:767-777.

Lofgren, C. S. 1986. History of imported fire ants in the United States. In *Fire Ants and Leafcutting Ants Biology and Management.* (Lofgren, C. S. and Vander Meer, R. K. Eds.), pp. 36-47. Westview Press, Boulder, CO.

Lord, J. C., Wraight, S. P., Daoust, R. A., and Roberts, D. W. 1988. Pathogenicity of *Beauveria bassiana* and *Zoophthora radicans* to the Coccinellid predators *Coleomegilla maculata* and *Eriopis connexas*. J. Invertebr. Pathol. 52:471-473.

Macleod, D. M. 1954. Investigations of the genera *Beauveria* Vuill. and *Tritirachium* Limber. Can. J. Bot. 32:818-819.

Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular cloning: a laboratory manual. Cold Springs Harbor Laboratory, Cold Springs Harbor, N. Y.

Matthew, J., Hawke, B. G., and Pankhurst, C. E. 1995. A DNA probe for identification of *Pythium irregulare* in soil. Mycol. Res. 99:579-584.

Maurer, P., Couteaudier, Y., Girard, P. A., Bridge, P. D., and Riba, G. 1997a. Genetic diversity of *Beauveria bassiana* and relatedness to host insect range. Mycol. Res. 101:159-164.

Maurer, P., Rejasse, A., Capy, P., Langin, T., and Riba, G. 1997b. Isolation of the transposable element *hupfer* from the entomopathogenic fungus *Beauveria bassiana* by insertion mutagenesis of the nitrate reductase structural gene. Mol. Gen. Genet. 256:195-202.

Maynard Smith, J., Smith, N. H., O'Rourke, M., and Spratt, B. G. 1993. How clonal are bacteria? Proc. Natl. Acad. Sci. USA 90:4383-4388.

McAlpin, C. E., and Mannarelli, B. 1995. Construction and characterization of a DNA probe for distinguishing strains of *Aspergillus flavus*. Appl. Environ. Microbiol. 61:1068-1072.

McCoy, C. W., Beavers, G. M. and Tarrant, C. A. 1985. Susceptibility of *Artipus floridanus* to different isolates of *Beauveria bassiana.* Florida Entomol. 68:402-409.

McCoy, C. W., Samson, R. A., and Boucias, D. G. 1988. Entomogenous fungi. In *Handbook of Natural Pesticides, Vol. 5, Microbial Insecticides, Part A, Entomogenous Protozoa and Fungi* (Ignoffo, C. M. Ed.), pp. 151-236. CRC Press, Boca Raton, FL.

McDonald, B. A., and Martinez, J. P. 1990. Restriction fragment length polymorphisms in *Septoria tritici* occur at a high frequency. Curr. Genet. 17: 133-138.

McDonald, B. A., and Martinez, J. P. 1991. DNA fingerprinting of the plant pathogenic fungus *Mycosphaerella graminicola* (anmorph *Septoria tritici*). Exp. Mycol. 15:146-158.

McDonald, B. A., and McDermott, J. M. 1993. Population genetics of plant pathogenic fungi. BioScience 43:311-319.

McDowell, J. M., Funderburk, J. E., Boucias, D. G., Gilreath, M. E., and Lynch, R. E. 1990. Biological activity of *Beauveria bassiana* against *Elasmopalpus lignosellus* (Lepidoptera: Pyralidae) on leaf substrates and soil. Environ. Entomol. 19:137-141.

McEachern, M. J., and Blackburn, E. H. 1994. A conserved sequence motif within the exceptionally diverse telomeric sequences of budding yeasts. Proc. Natl. Acad. Sci. USA 91:3453-3457.

Milgroom, M. G. 1995. Analysis of population structure in fungal plant pathogens. In *Disease Analysis through Genetics and Molecular Biology: Interdisciplinary Bridges to Improved Sorghum and Millet Crops*. (Leslie, J. F. and Frederiksen, R. A. Eds.), pp. 213-229. Iowa State University Press, Ames, Iowa.

Milgroom, M. G., and Lipari, S. E. 1993. Maternal inheritance and diversity of mitochondrial DNA in the chestnut blight fungus, *Cryphonectria parasitica*. Phytopathology 83:563-567.

Milgroom, M. G., Lipari, S. E., and Wang, K. 1992. DNA fingerprinting and analysis of population structure in the chestnut blight fungus, *Cryphonectria parasitica*. Genetics 131:297-306.

Moore, K. C., and Erlandson, M. A. 1988. Isolation of *Aspergillus parasiticus* and *Beauveria bassiana* from melanopline grasshoppers and a demonstration of their pathogenicity in *Melanoplus sanguinipes*. Can. Entomol. 120:989-991.

Mouyna, I., Renard, J. L., and Brygoo, Y. 1996. DNA polymorphism among *Fusarium oxysporum* f. sp. *elaeidis* populations from oil palm, using a repeated and dispersed sequence "Plam". Curr. Genet. 30:174-180.

Mugnai, L., Bridge, P. D., and Evans, H. C. 1989. A chemotaxonomic evaluation of the genus *Beauveria*. Mycol. Res. 92:199-209.

Neuveglise, C., Brygoo, Y., Vercambre, B., and Riba, G. 1994. Comparative analysis of molecular and biological characteristics of strains of *Beauveria brongniartii* isolated from insects. Mycol. Res. 98:322-328.

O'Brien, P. A. 1994. Molecular markers in Australian isolates of *Rhizoctonia solani*. Mycol. Res. 98:665-671.

O'Donnell, K., Kistler, H. C., Cigelnik, E., and Ploetz, R. C. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proc. Natl. Acad. Sci. USA 95:2044-2049.

Oi, D. H., Pereira, R. M., Stimac, J. L., and Wood, L. A. 1994. Field application of *Beauveria bassiana* for control of the red imported fire ant (Hymenoptera: Formicidae). J. Econ. Entomol. 87:623-630.

Paccola-Meirelles, L. D., and Azevedo, J. L. 1991. Genetic recombination by protoplast fusion in the Deuteromycete *Beauveria bassiana*. Rev. Brasil Genet. 17:15-18.

Pereira, R. M., Alves, S. B., and Stimac, J. L. 1993b. Growth of *Beauveria bassiana* in fire ant nest soil with amendments. J. Invertebr. Pathol. 62:9-14.

Pereira, R. M., and Roberts, D. W. 1990. Dry mycelium preparations of entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. J. Invertebr. Pathol. 56:39-46.

Pereira, R. M., Stimac, J. L., and Alves, S. B. 1993a. Soil antagonism affecting the dose-response of workers of the red imported fire ant, *Solenopsis invicta*, to *Beauveria bassiana* conidia. J. Invertebr. Pathol. 61:156-161.

Persson, Y., Erland, S., and Jansson, H. B. 1996. Identification of nematode-trapping fungi using RFLP analysis of the PCR-amplified ITS region of ribosomal DNA. Mycol. Res. 100:531-534.

Petch, T. 1926. Studies in entomogenous fungi. VIII. Notes on *Beauveria*. Trans. Bri. Mycol. Soc. 10:244-271.

Pfeifer, T. A., Hegedus, D. D., and Khachatourians, G. G. 1992. The mitochondrial genome of the entomopathogenic fungus *Beauveria bassiana*: analysis of the ribosomal RNA region. Can. J. Microbiol. 39:25-31.

Pfeifer, T. A., and Khachatourians, G. G. 1989. Isolation and characterization of DNA from the entomopathogen *Beauveria bassiana*. Exp. Mycol. 13:392-402.

Pfeifer, T. A., and Khachatourians, G. G. 1992. *Beauveria bassiana* protoplast regeneration and transformation using electropotation. Appl. Microbiol. Biotechnol. 38:376-381.

Pfeifer, T. A., and Khachatourians, G. G. 1993. Electrophoretic karytype of the entomopathgenic Deuteromycete *Beauveria bassiana*. J. Invertebr. Pathol. 61: 231-235.

Pingel, R. L., and Lewis, L. C. 1996. The fungus *Beauveria bassiana* (Balsamo) Vuillemin in a corn ecosystem: its effect on the insect predator *Coleomegilla maculata* De Geer. Biol. Contr. 6:137-141.

Poprawski, T. J., Riba, G., Jones, W. A., and Aioun, A. 1988. Variation in isoesterase profiles of geographical population of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) isolated from *Sitona weevils* (Coleoptera: Curculionidae). Environ. Entomol. 17:257-269.

Poprawski, T. J., Robert, P.-H., Majchrowicz, I., and Boivin, G. 1985. Susceptibility of *Delia antiqua* (Diptera: Anthomyiidae) to eleven isolates of entomopathogenic Hyphomycetes. Environ. Entomol. 14:557-561.

Poprawski, T. J., and Yule, W. N. 1991. Incidence of fungi in natural populations of *Phyllophaga* spp. and susceptibility ot *Phyllophaga anxia* (LeConte) (Col., Scarabaeidae) to *Beauveria bassiana* and *Metarhizium anisopliae* (Deuteromycotina). J. Appl. Entomol. 112:359-365.

Powell, W. A., and Kistler, H. C. 1990. In vivo rearrangement of foreign DNA by *Fusarium oxysporum* produces linear self-replicating plasmids. J. Bacteriol. 172:3163-3171.

Prior, C., Jollands, P., and Le Patourel, G. 1988. Infectivity of oil and water formulations of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) to the cocoa weevil pest *Pantorhytes plutus* (Coleoptera: Curculionidae). J. Invertebr. Pathol. 52:66-72.

Pujol, C., Joly, S., Lockhart, S. R., Noel, S., Tibayrenc, M., and Sol, D., R. 1997. Parity among the randomly amplified polymorphic DNA method, multilocus enzyme electrophoresis, and Southern blot hybridization with the moderately repetitive DNA probe Ca3 for fingerprinting *Candida albicans*. J. Clin. Microbiol. 35:2348-2358.

Pujol, C., Reynes, J., Renaud, F., Raymond, M., and Tibayrenc, M., 1993. The yeast *Candida albicans* has a clonal mode of reproduction in a population of infected HIV positive patients. Proc. Natl. Acad. Sci. USA 90:9456-59.

Rakotonirainy, M. S., Dutertre, M., Brygoo, Y., and Riba, G. 1991. RNA sequence comparison of *Beauveria bassiana, Tolypocladium cylindrosporum*, and *Tolypocladium extinguens.* J. Invertebr. Pathol. 57:17-22.

Riba, G., Katagiri, K., and Kawakami, K. 1982. Preliminary studies on the susceptibility of the silkworm *Bombyx bori* (Lepidoptera: Bombycidae) to some entomogenous hyphomycetes. Appl. Entomol. Zool. 17:238-243.

Riba, G., Poprawski, T. J., and Mariania, J. 1986. Isoesterase variability among geographical populations of *Beauveria bassiana* (fungi Imperfecti) isolated from Miridae. In *Fundamental and Applied Aspects of Invertebrate Pathology* (Samsom, R. A., Vlak, J. M. and Peters, D., Eds.), pp. 205-209. Foundation of the Fourth International Colloquium of Invertebrate Pathology: Wageningen, The Netherlands.

Richards, E. J., and Ausubel, F. M. 1988. Isolation of a higher eukaryotic telomere from *Arabidopsis thaliana.* Cell 53:127-136.

Roberts D. W., and Hajek, A. E. 1992. Entomopathogenic fungi as bioinsecticides. In *Frontiers in Industrial Mycology* (Leatham, G. F., Ed.), pp. 201-236. Chapman & Hall, New York.

Roberts, D. W., LeBrun, R. A., and Semel, M. 1981. Control of the Colorado potato beetle with fungi. In *Advances in potato pest management.* (Lashomb, H., and Casagrande, R. A., Eds.), pp. 119-137. Hutchinson Ross, stroudsburg, PA.

Rosewich, U. L., and McDonald, B. A. 1994. DNA Fingerprinting in fungi. Methods Mol. Cell. Biol. 5:41-48.

Samsinakova, A., and Kalaova, S. 1983. The influence of a single-spore isolate and repeated subculturing on the pathogenicity of conidia of the entomophagous fungus *Beauveria bassiana.* J. Invertebr. Pathol. 42:156

Samson, R. A. 1995. Constraints associated with taxonomy of biocontrol fungi. Can. J. Bot. 73 (Suppl.1):S83-S88.

Samson, R. A., and Evans, H. C. 1982. Two new *Beauveria* spp. from South America. J. Invertebr. Pathol. 9:93-97.

Sanchez-Alonso, P., Valverde, M. E., Paredes-Lopez, O., and Guzman, P. 1996. Detection of genetic variation in *Ustilago maydis* strains by probes derived from telomeric sequences. Microbiology 142:2931-2936.

Scherer, S., and Stevens, D. A. 1988. A *Candida albicans* dispersed, repeated gene family and its epidemiologic applications. Proc. Natl. Acad. Sci. USA 85: 1452-1456.

Schmid-Berger, N., Schmid, B., and Barth, G. 1994. *Yltl,* a highly repetitive retrotransposon in the genome of the dimorphic fungus *Yarrowia lipolytica*. J. Bacteriol. 176:2477-2482.

Shih, H. L., Lin, C. P., Liou, R. F., and Tzean, S. S. 1995. Complete nucleotide sequence of *Beauveria bassiana* 5.8s rRNA coding gene and flanking internal transcribed spacers. DNA Sequence 5:381-383.

Shimizu, S. 1987. Protoplast fusion of insect pathogenic fungi. In *Biotechnology in Invertebrate Pathology and Cell Culture.* (Maramorosh, K., Ed.), pp 401-414. Academic Press, San Diego.

Shimizu, S., and Aizawa, K. 1988. Serological classification of *Beauveria bassiana.* J. Invertebr. Pathol. 52:348-353.

Smith, M. L., and Anderson, J. B. 1989. Restriction fragment length polymorphisms in mitochondrial DNAs of *Armillaria*: identification of North American biological species. Mycol. Res. 93:247-256.

Spitzer, E. D., and Spitzer, S. G. 1992. Use of a dispersed repetitive DNA element to distinguish clinical isolates of *Cryptococcus neoformans*. J. Clin. Microbiol. 30:1094-1097.

Steinkraus, D. C., Geden, C. J., and Rutz, D. A. 1991. Susceptibility of lesser mealworm (Coleoptera: Tenebrionidae) to *Beauveria bassiana* (Moniliales: Moniliaceae): effects of host stage, substrate, formulation, and host passage. J. Med. Entomol. 28:314-321.

Steinkraus, D. C., Geden, C. J., Rutz, D. A., and Kramer, J. P. 1990. First report of the natural occurrence of *Beauveria bassiana* (Moniliales: Moniliaceae) in *Musca domestica* (Diptera: Muscidae). J. Med. Entomo. 27:309-312.

Steinkraus, D. C., and Tuqwell, N. P. 1997. *Beauveria bassiana* (Deuteromycotina: Moniliales) effects on *Lygus lineolaris* (Hemiperat: Miridae). J. Entomol. Sci. 32:79-90.

Stimac, J. L., and Alves, S. B. 1994. Ecology and biological control of fire ants. In *Pest Management in the Subtropics: Biological control – A Florida Perspective.* (Rosen, D., Bennett, F. D. and Capinera J. L., Eds), pp353-380. Intercept Limited, UK.

Stimac, J. L., Alves, S. B., and Camargo, M. T. V. 1987. Suscetibilidade de *Solenopsis* spp. a diferentes especies de fungos entomopatognicos. An Soc. Entomol. Bras. 16:377-387. (in Portuguese).

Stimac, J. L., Pereira, R. M., Alves, S. B., and Wood, L. A. 1993a. *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycetes) applied to laboratory colonies of *Solenopsis invicta* Buren (Hymenoptera: Formicidae) in soil. J. Econ. Entomol. 86:348-352.

Stimac, J. L., Pereira, R. M., Alves, S. B., and Wood, L. A. 1993b. Mortality in laboratory colonies of S*olenopsis invicta* Buren (Hymenoptera: Formicidae) treated with *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycetes). J. Econ. Entomol. 86:1083-1087.

St. Leger, R. J., Allee, L. L., May, B., Staples, R. C., and Roberts, D. W. 1992. World-wide distribution of genetic variation among isolates of *Beauveria* spp. Mycol. Res. 96:1007-1015.

Studdert, J. P., and Kaya, H. K. 1990. Water potential, temperature, and clay-coating on survival of *Beauveria bassiana* conidia in a loam and peat soil. J. Invertebr. Pathol. 55:417-420.

Talmadge, H. E. 1980. Statement on the fire ant problem. Proc. Tall Timbers Conf. Ecol. Anim. Control. Hab. Manag. 7:9-12.

Tantaoui, A., Ouinten, M., Geiger, J. P., and Fernandez, D. 1996. Characterization of a single clonal lineage of *Fusarium oxysporum* f. sp. *albedinis* causing bayoud disease of date palm in Morocco. Phytopathology 86:787-792.

Tibayrenc, M., Kjellberg, F., Arnaud, J., Oury, B., and Breniere, S. F. 1991. Are eukaryotic microorganisms clonal or sexual? A population genetics vantage. Proc. Natl. Acad. Sci. USA 88:5129-5133.

Tibayrenc, M., Kjellberg, F., and Ayala, F. J. 1990. A clonal theory of parasitic protozoa: the population structure of *Entamoeba, Giardia, Leishmania, Naegleria, Plasmodium, Trichomonas* and *Trypanosoma*, and its medical and taxonomical consequences. Proc. Natl. Acad. Sci. USA 87:2414-2418.

Timberlake, W. E. 1978. Low repetitive DNA content in *Aspergillus nidulans*. Science 202:973-975.

Todorova, S. I., Cote, J. C., Martel, P., and Coderre, D. 1994. Heterogeneity of two *Beauveria bassiana* strains revealed by biochemical tests, protein profiles and bio-assays on Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) and *Coleomegilla maculata lengi* Timberlake (Coleoptera: Coccinellidae) Larvae. Entomophaga 39:159-169.

Todorova, S. I., Cote, J. C., and Coderre, D. 1996. Evaluation of the effects of two *Beauveria bassiana* (Balsamo) Vuillemin strains on the development of *Coleomegilla maculata lengi* Timberlake (Col., Coccinellidae). J. Appl. Entomol. 120:159-163.

Vandenberg, J. D. 1996. Standardized bioassay and screening of *Beauveria bassiana* and *Paecilomyces umosoroseus* against the Russian wheat aphid (Homoptera: Aphididae). J. Econ. Entomol. 89:1418-1423.

Varela, A., and Morales, E. 1996. Characterization of some *Beauveria bassiana* isolates and their virulence toward the coffee berry borer *Hypothenemus hampei*. J. Invertebr. Pathol. 67:147-152.

Varma, A., and Kwon-Chung, K. J. 1989. Restriction fragment polymorphism in mitochondrial DNA of *Cryptococcus neoformans*. J. Gen. Microbiol. 135: 3353-3362.

Varma, A., and Kwon-Chung, K. J. 1992. DNA probe for strain typing of *Cryptococcus neoformans*. J. Clin. Microbiol. 30:2960-2967.

Viaud, M., Couteaudier, Y., Levis, C., and Riba, G. 1996. Genome organization in *Beauveria bassiana:* electrophoretic karyotype, gene mapping, and telomeric fingerprint. Fung. Genet. Biol. 20:175-183.

Viaud, M., Couteaudier, Y., and Riba, G. 1998 Molecular analysis of hypervirulent somatic hybrids of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria sulfurescens*. Appl. Environ. Microbiol. 64:88-93.

Von Arx, J. A. 1986. Tolypocladium, a synonym of *Beauveria.* Mycotaxon 25:153-158.

Vuillemin, P. 1912. *Beauveria*, nouveau genre de Verticilliacees. Bull. Soci. Botan. Fran. 29:34-40.

Ward, E., and Akrofi, A. Y. 1994. Identification of fungi in the *Gaeumannomyces-Phialphora* complex by RFLPs of PCR- amplified ribosomal DNAs. Mycol. Res. 98: 219-224.

White, T. J., Bruns, T. D., Lee, S. B., and Taylor, J. W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols, a Guide to Methods and Applications.* (Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J., Eds), pp. 315-322. Academic Press: San Diego.

Wingfield, B. D., Harrington, T. C., and Steimel, J. 1996. A simple method for detection of mitochondrial DNA polymorphisms. Fungal Genet. News.43:56-60.

Wraight, S. P., Carruthers, R. I., Bradley, C. A., Jaronski, S. T., Lacey, L. A., Wood, P., and Galaini-Wraight, S. 1998. Pathogenicity of the entomopathogenic fungi *Paecilomyces* spp. and *Beauveria bassiana* against the silverleaf whitefly, *Bemisia argentifolii*. J. Invertebr. Pathol. 71: 217-226.

Wright, J. E. 1992. Whiteflies: development of Naturalis-L, a biorational mycoinsecticide for control. In *Proceedings Beltwide Cotton Conferences,* vol. 2. (Herber D. J. and Richter D. A. Eds). pp. 887-888. National Cotton Council of America, Memphis, TN.

Wright, J. E., and Chandler, L. D. 1991. Laboratory evaluation of the entomopathogenic fungus, *Beauveria bassiana* against the boll weevil (Curculionidae: Coleoptera). J. Invertebr. Pathol. 58: 448-449.

Wright, J. E. and Chandler, L. D. 1992. Development of a biorational mycoinsecticide: *Beauveria bassiana* conidial formulation and its application against the boll weevil populations (Curculionidae: Coleoptera). J. Econ. Entomol. 85: 1130-1135.

Wright, J. E., and Chandler, L. D. 1993. Control of the boll weevil (Curculionidae: Coleoptera) with Naturalis-L: a mycoinsecticide. J. Econ. Entomol. 86: 1355-1358.

Xia, J. Q., Correl, J. C., Lee, F. N., Marchetti, M. A., and Rhoads, D. D. 1993. DNA fingerprinting to examine microgeographic variation in the *Magnaporthe grisea* (Pyricularia grisea) population in two rice fields in Arkansas. Phytopathology 83: 1029-1035.

Zakian V. A. 1989. Structure and fusion of telomeres. Ann. Rev. Genet. 23: 579-604.

BIOGRAPHICAL SKETCH

Yong Zeng was born on April 9, 1964, in Nanning, Guangxi, P. R. China. He entered the Second Nanning High School in August 1978. After graduation in June 1982, he entered Beijing Agricultural University and received a Bachelor of Science degree in July 1986 and a Master of Science degree in July 1989 with a major in entomology. Then, he started to work as a research associate in Guangdong Entomological Institute, Guangzhou. In November 1992, he worked in the Agrochemical Division, Rhone-Poulenc Asia Limited. In June 1994, he started a doctoral program in the Entomology and Nematology Department at the University of Florida. He married Jennifer Zhang in 1998.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Jerry L. Stimac

Jerry L. Stimac, Chair
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James E. Maruniak

James E. Maruniak, Cochair
Associate Professor of Entomology and
Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Harold C. Kistler

Harold C. Kistler
Professor of Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James W. Kimbrough

James W. Kimbrough
Professor of Plant Pathology

This dissertation was submitted to the Graduate Faculty of College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 1999

Rachel B Shireman

Dean, College of Agriculture

Dean, Graduate School